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Benthic Analysis Training Manual

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Benthic Analysis

Training Manual

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A lecture manual for technicians and biologists who have responsibility for collection, identification and interpretation of findings of benthic communities in surveillance of the aquatic environment. This is intended as an introductory approach in benthic community analysis.

Topics cover and emphasize benthic macroinvertebrate communities in fresh water, including sampling considerations, taxonomic analysis, sorting techniques, sampling handling, and data presentation and interpretation.

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BENTHIC ANALYSES

This course is for technicians and biologists who have responsibility for collection, identification and interpretation of findings of benthic communities in surveillance of the aquatic environment. This is an introductory course in benthic community analysis.

After successfully completing the course, the student will be able to plan, conduct and evaluate benthic monitoring programs.

The training consists of classroom instruction and activities, laboratory studies and field experience.

Course coverage emphasizes benthic macroinvertebrate communities in fresh water, including sampling considerations. taxonomic analysis, sorting techniques, sampling handling, and data presentation and interpretation.

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BENTHIC COMMUNITIES (MARINE)

I INTRODUCTION

A The term community in the ecological sense includes all of the populations of animals and plants occupying a given area. The community and the nonliving environment function together as an ecological system or ecosystem.

B The community cannot exist without the cycling of materials and the flow of energy in the ecosystem.

C The community concept is important in ecological theory because it emphasizes the fact that diverse organisms usually live together in an orderly manner.

D Since organisms in salt water (or in any other natural habitat) are not arranged in taxonomic or systematic orders, classification on an ecological basis as one of the following is useful:

1 On major niches or position in energy or food chain

  a Producers

  b Consumers

  c Decomposers

2 On their mode of life

  a Benthos

  b Periphyton

  c Plankton

  d Nekton

  e Neuston

3 On region or subhabitat (see Figure 1)

  a Intertidal

  b Subtidal

  c Lower Neritic

  d Bathyal

  e Abyssal

  f Hadal

E Among benthic organisms the biotic factors of the environment are manifested in predacity, competition for food and space, and materialisms.

F The zonation of varied and important groups of plant-like sessile animals on the sea bottom is often as striking as the zonation of trees on a mountain and similarly provides shelter for small organisms.

G Because of their stability benthic organisms provide a basis for the classification of zones.

II THE BENTHIC HABITAT

A Distinct faunas in marine environments depend on the physical characteristics of the environments, the ecological activities of the organisms, the geological history of the region and the biology of the species. On this basis the following types of assemblages and environments can be generalized:

1 Intertidal and rocky shores.

2 Intertidal sand beaches and flates to 10 m.

3 Low salinity lagoons and mangroves.

4 Nearshore, sand and sand-mud, 10-30 m.

5 Intermediate shelf to 65 m.

6 Outer shelf to 130 m.

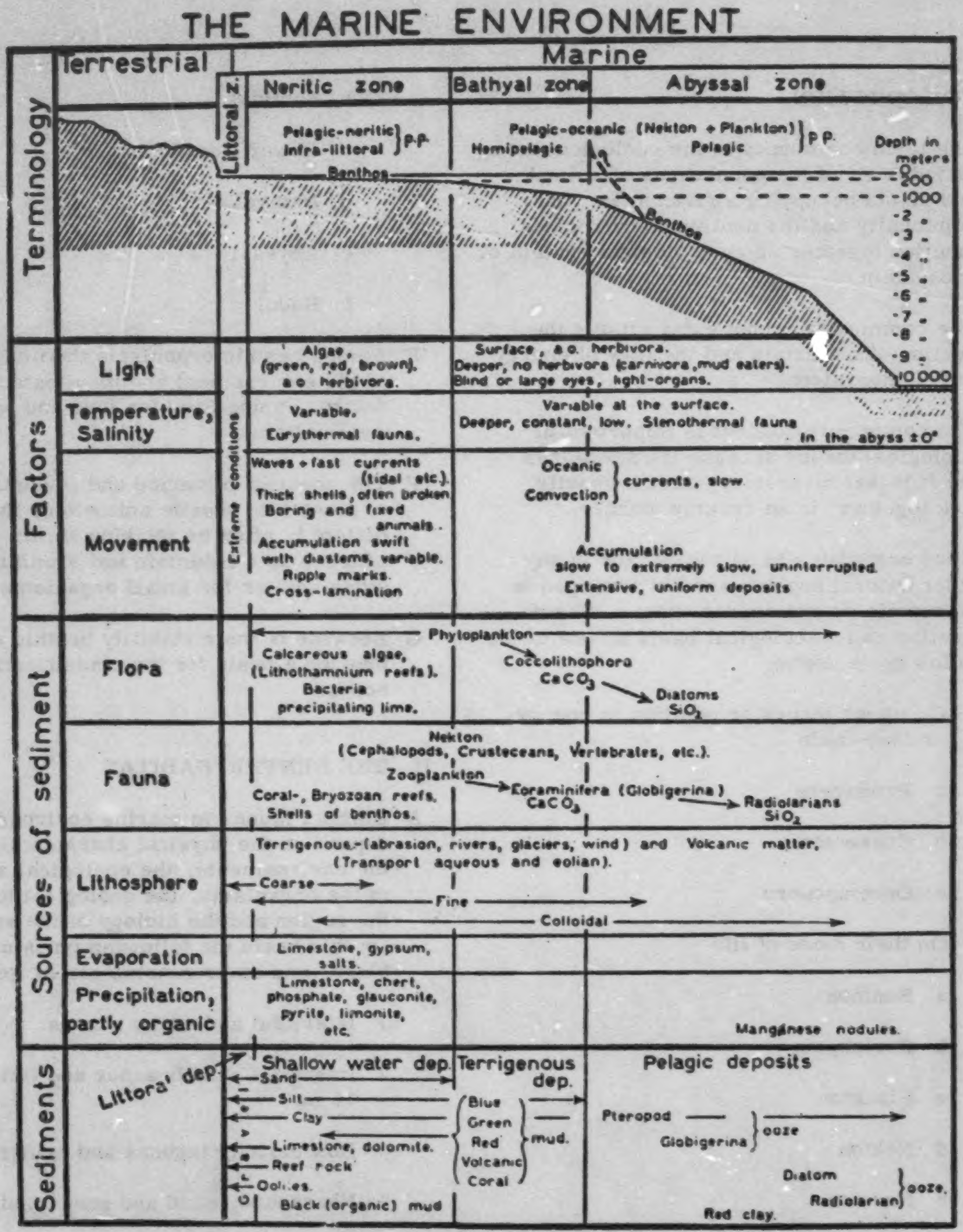
THE MARINE ENVIRONMENT
Terminology
Terrestrial
Marine
Littoral z.
Neritic zone
Bathyal zone
Abyssal zone
Pelagic-neritic
Infra-littoral
P.P.
Pelagic-oceanic (Nekton + Plankton)
Hemipelagic
Pelagic
P.P.
Benthos
Benthos
Depth in meters
0
200
1000
2
3
4
5
6
7
8
9
10 000
Factors
Light
Algae, (green, red, brown). a.o. herbivora.
Surface, a.o. herbivora.
Deeper, no herbivora (carnivora, mud eaters).
Blind or large eyes, light-organs.
Temperature, Salinity
Variable.
Eurythermal fauna.
Variable at the surface.
Deeper, constant, low. Stenothermal fauna
In the abyss ±0°
Extreme conditions
Movement
Waves + fast currents (tidal etc.).
Thick shells, often broken
Boring and fixed animals.
Accumulation swift with diastems, variable.
Ripple marks.
Cross-lamination
Oceanic
Convection
currents, slow.
Accumulation slow to extremely slow, uninterrupted.
Extensive, uniform deposits
Sources of sediment
Flora
Phytoplankton
Calcareous algae, (Lithothamnium reefs).
Bacteria precipitating lime.
Coccolithophora CaCO3
Diatoms SiO2
Fauna
Nekton (Cephalopods, Crustaceans, Vertebrates, etc.).
Zooplankton
Foraminifera (Globigerina) CaCO3
Radiolarians SiO2
Coral-, Bryozoan reefs.
Shells of benthos.
Lithosphere
Terrigenous (abrasion, rivers, glaciers, wind) and Volcanic matter,
(Transport aqueous and eolian).
Coarse
Fine
Colloidal
Evaporation
Limestone, gypsum, salts.
Precipitation, partly organic
Limestone, chert, phosphate, glauconite, pyrite, limonite, etc.
Manganese nodules.
Sediments
Littoral dep.
Shallow water dep.
Terrigenous dep.
Pelagic deposits
Gravel
Sand
Silt
Clay
Limestone, dolomite.
Reef rock
Oolites.
Black (organic) mud
Blue
Green
Red
Volcanic
Coral
mud.
Pteropod
Globigerina
ooze
Diatom
Radiolarian
ooze.
Red clay.

Figure 1. THE ENVIRONMENTS OF SEDIMENTATION IN THE OCEAN

Table 1. SIZE CLASSIFICATION OF SEDIMENTARY PARTICLES

As frequent reference will be made to materials of various grain sizes, the following table shows the most used size classifications (grade scales)

Diameters in millimeters

Wentworth			Atterberg		
Boulder Cobble	Above 256 256-64		Block Cobble	2000-200 200-20	Gravel } Psephite
Pebble Granule	64-4 4-2		Pebble	20-2	
Very coarse sand Coarse sand Medium sand Fine sand Very fine sand	2-1 1-½ ½-¼ ¼-⅛ ⅛-1/16	2-1 1-0.5 0.5-0.25 0.25-0.125 0.125-0.0625	Coarse sand Fine sand	2-0.2 0.2-0.02	Psammite (arenaceous)
Silt Clay	1/16-1/256 Below 1/256	0.0625-0.004 Below 0.004.	Silt Clay	0.02-0.002 Below 0.002	Pelite or lutite

The finest fractions of sediments are frequently called "clay." But as this word has a definite mineralogical implication the terms "pelite" or "lutite" or "lutum" are used by some authors to denote all particles smaller than the sand fraction, whether consisting of clay minerals, calcite, or any other mineral. The word "ooze" signifies a fine deposit composed principally of the shells and debris of pelagic organisms. By "mud" the marine geologist means all fine-grained deposits of a more or less plastic nature in moist condition.

For a more extensive review of this and other problems of sedimentary petrography the reader may consult Krumbein and Pettijohn (1938), Pettijohn (1949), or other manuals of petrography and also Twenhofel's books.

From Kuenen, P.H. Marine Geology. 1950.

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a clay bottom or

b sand bottom (depth figures are approximation)

7 Basins and troughs to 1500 m.

8 Upper (inner) Continental Slope.

9 Middle Continental Slope.

10 Lower (outer) Continental Slope.

11 Abyssal basins to 10,000 m.

12 Hadal basins to 13,000 m.

B Investigations of marine bottom communities, with relatively few exceptions have been concerned with describing the aggregations of species of infaunal invertebrates along the broad areas of differing sedimentary factors.

C An investigation of the temporal sequences and biotic successions in the fouling of artifacts may be a prime feature in the detection of pollution.

D In shore the relationship between population density and substratum type is quite striking. This is brought about by rain wash, stream erosion, aeolian deposits and glacial action.

E Marine sediments on and in which the benthos lives are derived from a great number of different sources

- 1 Sources
    - a Skeletons and tests of organisms
    - b Decomposable organic matter ($1\ kg/m^2/yr.$ in coastal waters)
    - c Precipitates: lime, iron, Mn., etc.
    - d Coastal and bottom erosion: waves, currents, etc.
    - e Rivers (Miss. R. $1\ km^3$ sand and silt/yr)
    - f Glaciers and ice
    - g Weathering on the sea floor
- 2 Media of transport
    - a The atmosphere
        - 1) Meteoric dust (10 to 20 million particles/day)
        - 2) Volcanos: pumice, ash, etc.
        - 3) Offshore winds; dust, sand
    - b Sediment slumping and currents (settling rates)
    - c Horizontal currents
    - d Currents and wave turbulence
    - e Rhythmic accumulations

F The macroscopic fauna of sandy beaches is composed mostly of burrowers.

G The microscopic fauna of sandy beaches is highly specialized and depends largely on the composition of the substratum and the percent composition of sand grain size.

H In the neritic zone the benthos are consumers and exhibit marked zonation because of the large number of distinct sessile forms. They are distinct for each of the neritic zones.

I The unequal distribution of animal mass in shallow waters is the result of intertwined biological and physical factors in the bottom, some of which involve aerobic and anaerobic exchanges with the environment, but many of which are not understood.

J The burrowing habit is more common in muddy sand than in sand or solid rock.

K The variety of organisms on the bottom is greatest where measurable light can reach to the bottom and where plankton production is high.

L The eulittoral zone gives rise to many communities because it is greatly varied with regard to type of substratum and also to character of shoreline and degree of exposure.

M In deeper water, 2000 m or more, gravels, sands and silts from the land are replaced by pelagic oozes or red clay. The former are important as a substratum and because of their organic matter content.

N On the level bottoms of the sea live 2 ecologically distinct groups: the eipfauna comprising 3% of all species of animals (more than 4/5 of all bottom dwellers) and the infauna making up 11% of known species. (less than 1/5 of all bottom dwellers).

O Rocky shores from outcrops, boulders, etc. present characteristic faunas on their slopes, in crevices and in pools, communities whose composition is also dependent on vagueries of the tides.

P Benthic communities are irregularly spaced: the same type of level bottom substrata at similar depths in widely spread regions support paralleled groups of invertebrates.

Q The bottom fishes as soles, plaice, halibut, founders, rays, sculpin, etc., depend to a great extent on consistent productivity of the bottom on which they live for food, shelter and reproduction.

R Deep sea benthic animals are mainly mud-dwelling forms variously adapted to this mode of life. The deepest fauna in the most widely distributed; the poverty of individuals being apparently related to distance from the shore.

S Much of the animal life on the deep sea is endemic not merely an extension of eurybathic forms. This fauna has a relatively small number of species in proportion to the number of genera.

T The abyssal and hadal zones are regions of relatively uniform conditions in terms of pressure, temperature and light. The communities in these areas are correspondingly similar while in many respects and depend on the productivity of the euphotic zone.

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This outline was prepared by D. J. Zinn, Professor of Zoology, Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island.

Descriptors: Aquatic Life, Benthos, Benthic fauna, Benthic flora, Biological communities, Ecological distribution, Marine biology, and Oceans.

USING BENTHIC BIOTA IN WATER QUALITY EVALUATION

I BENTHOS ARE ORGANISMS GROWING ON OR ASSOCIATED PRINCIPALLY WITH THE BOTTOM OF WATERWAYS

Benthos is the noun.

Benthonic, benthal and benthic are adjectives.

II THE BENTHIC COMMUNITY

A Composed of a wide variety of life forms that are related because they occupy "common ground"--substrates of oceans, lakes, streams, etc. Usually they are attached or have relatively weak powers of locomotion. These life forms are:

1 Bacteria

A wide variety of decomposers work on organic materials, breaking them down to elemental or simple compounds.

2 Algae

Photosynthetic plants having no true roots, stems, and leaves. The basic producers of food that nurtures the animal components of the community.

3 Flowering Aquatic Plants (Pondweeds)

The largest flora, composed of complex and differentiated tissues. May be emersed, floating, submersed according to habit.

4 Microfauna

Animals that pass through a U.S. Standard Series No. 30 sieve, but are retained on a No. 100 sieve. Examples are rotifers and microcrustaceans. Some forms have organs for attachment to substrates, while others burrow into soft materials or occupy the interstices between rocks, floral or faunal materials.

5 Meiofauna

Meiofauna occupy the interstitial zone (like between sand grains) in benthic and hyporheic habitats. They are intermediate in size between the microfauna (protozoa and rotifers) and the macrofauna (insects, etc.). They pass a No. 30 sieve (0.5 mm approximately). In freshwater they include nematodes, copepods, tardigrades, naiad worms, and some flat worms. They are usually ignored in freshwater studies, since they pass the standard sieve and/or sampling devices.

6 Macrofauna (macroinvertebrates)

Animals that are retained on a No. 30 mesh sieve (0.5 mm approximately). This group includes the insects, worms, molluscs, and occasionally fish. Fish are not normally considered as benthos, though there are bottom dwellers such as sculpins and darters.

B It is a self-contained community, though there is interchange with other communities. For example: Plankton settles to it, fish prey on it and lay their eggs there, terrestrial detritus leaves are added to it, and many aquatic insects migrate from it to the terrestrial environment for their mating cycles.

C It is stationary water quality monitor. The low mobility of the biotic components requires that they "live with" the quality changes of the over-passing waters. Changes imposed in the long-lived components remain visible for extended periods, even after the cause has been eliminated. Only time will allow a cure for the community by drift, reproduction, and recruitment from the hyporheic zone.

D Between the benthic zone (substrate/water interface) and the underground water table is the hyporheic zone. There is considerable interchange from one zone to another.

III HISTORY OF BENTHIC OBSERVATIONS

A Ancient literature records the vermin associated with fouled waters.

B 500-year-old fishing literature refers to animal forms that are fish food and used as bait.

C The scientific literature associating biota to water pollution problems is over 100 years old (Mackenthun and Ingram, 1964).

D Early this century, applied biological investigations were initiated.

1 The entrance of state boards of health into water pollution control activities.

2 Creation of state conservation agencies.

3 Industrialization and urbanization.

4 Growth of limnological programs at universities.

E A decided increase in benthic studies occurred in the 1950's and much of today's activities are strongly influenced by developmental work conducted during this period. Some of the reasons for this are:

1 Movement of the universities from "academic biology" to applied pollution programs.

2 Entrance of the federal government into enforcement aspects of water pollution control.

3 A rising economy and the development of federal grant systems.

4 Environmental Protection Programs are a current stimulus.

IV WHY THE BENTHOS?

A It is a natural monitor

B The community contains all of the components of an ecosystem.

1 Reducers
   a bacteria
   b fungi

2 Producers (plants)

3 Consumers

   a Detritivores and bacterial feeders

   b Herbivores

   c Predators

C Economy of Survey

1 Manpower

2 Time

3 Equipment

D Extensive Supporting Literature

E Advantages of the Macrobenthos

1 Relatively sessile

2 Life history length

3 Fish food organisms

4 Reliability of Sampling

5 Dollars/information

6 Predictability

7 Universality

F "For subtle chemical changes, unequivocal data, and observations suited to some statistical evaluation will be needed. This requirement favors the macrofauna as a parameter. Macroinvertebrates are easier to sample reproductively than other organisms, numerical estimates are possible and taxonomy needed for synoptic investigations is within the reach of a non-specialist." (Wuhrmann)

G "It is self-evident that for a multitude of non-identifiable though biologically active changes of chemical conditions in rivers, small organisms with high physiological differentiation are most responsive. Thus the small macroinvertebrates (e.g. insects) are doubtlessly the most sensitive organisms for demonstrating unspecified changes of water chemistry called 'pollution'. Progress in knowledge on useful autecological properties of organisms or of transfer of such knowledge into bioassay practice has been very small in the past. Thus, the bioassay concept (relation of organisms in a stream to water quality) in water chemistry has brought not much more than visual demonstration of a few overall chemical effects. Our capability to derive chemical conditions from biological observations is, therefore, almost on the same level as fifty years ago. In the author's opinion it is idle to expect much more in the future because of the limitations inherent to natural bioassay systems (relation of organisms in a stream to water quality)." (Wuhrmann)

V REACTIONS OF THE BENTHIC MACROINVERTEBRATE COMMUNITY TO PERTURBATION

A Destruction of Organism Types

1 Beginning with the most sensitive forms, pollutants kill in order of sensitivity until the most tolerant form is the last survivor. This results in a reduction of variety or diversity of organisms.

2 The generalized order of macroinvertebrate disappearance on a sensitivity scale below pollution sources is shown in Figure 2.

Water Quality Deteriorating ↓		↑ Water Quality Improving
	Stoneflies Mayflies Caddisflies Amphipods Isopods Midges Oligochaetes	

As water quality improves, these tend to reappear in the same order.

B The Number of Survivors Increase

1 Competition and predation are reduced between different species.

2 When the pollutant is a food (plants, fertilizers, animals, organic materials).

C The Number of Survivors Decrease

1 The material added is toxic or has no food value.

2 The material added produces toxic conditions as a byproduct of decomposition (e.g., large organic loadings produce an anaerobic environment resulting in the production of toxic sulfides, methanes, etc.)

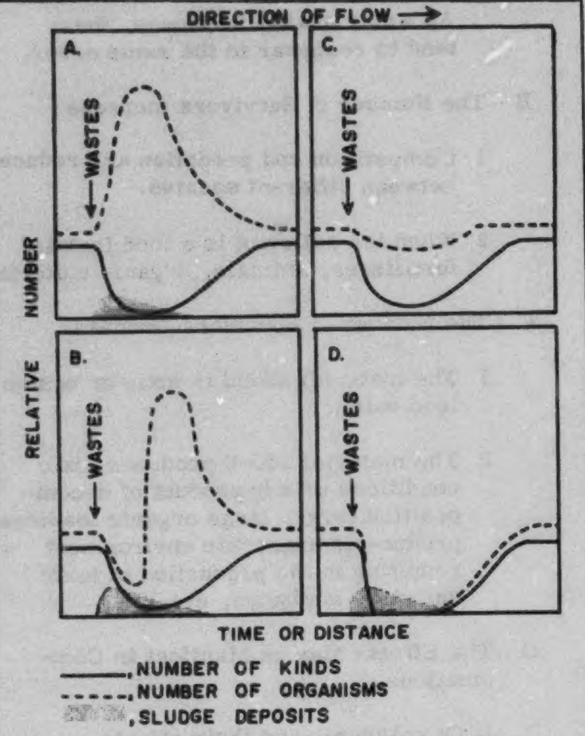
D The Effects May be Manifest in Combinations

1 Of pollutants and their effects.

2 Vary with longitudinal distribution in a stream. (Figure 1)

E Tolerance to Enrichment Grouping (Figure 2)

Flexibility must be maintained in the establishment of tolerance lists based on the response of organisms to the environment because of complex relationships among varying environmental conditions. Some general tolerance patterns can be established. Stonefly and mayfly nymphs, hellgrammites, and caddisfly larvae represent a grouping (sensitive or intolerant) that is generally quite sensitive to environmental changes. Blackfly larvae, scuds, sowbugs, snails, fingernail clams, dragonfly and damselfly naiads, and most kinds of midge larvae are facultative (or intermediate) in tolerance. Sludge-worms, some kinds of midge larvae (bloodworms), and some leeches



Four basic responses of bottom animals to pollution. A. Organic wastes eliminate the sensitive bottom animals and provide food in the form of sludges for the surviving tolerant forms. B. Large quantities of decomposing organic wastes eliminate sensitive bottom animals and the excessive quantities of byproducts of organic decomposition inhibit the tolerant forms; in time, with natural stream purification, water quality improves so that the tolerant forms can flourish, utilizing the sludges as food. C. Toxic materials eliminate the sensitive bottom animals; sludge is absent and food is restricted to that naturally occurring in the stream, which limits the number of tolerant surviving forms. Very toxic materials may eliminate all organisms below a waste source. D. Organic sludges with toxic materials reduce the number of kinds by eliminating sensitive forms. Tolerant survivors do not utilize the organic sludges because the toxicity restricts their growth.

Figure 1

are tolerant to comparatively heavy loads of organic pollutants. Sewage mosquitoes and rat-tailed maggots are tolerant of anaerobic environments for they are essentially air-breathers.

F Structural Limitations

1 The morphological structure of a species limits the type of environment it may occupy.

a Species with complex appendages and exposed complicated respiratory structures, such as stonefly nymphs, mayfly nymphs, and caddisfly larvae, that are subjected to a constant deluge of setteable particulate matter soon abandon the polluted area because of the constant preening required to maintain mobility or respiratory functions; otherwise, they are soon smothered.

b Benthic animals in depositing zones may also be burdened by "sewage fungus" growths including stalked protozoans. Many of these stalked protozoans are host specific.

2 Species without complicated external structures, such as bloodworms and sludgeworms, are not so limited in adaptability.

a A sludgeworm, for example, can burrow in a deluge of particulate organic matter and flourish on the abundance of "manna."

b Morphology also determines the species that are found in riffles, on vegetation, on the bottom of pools, or in bottom deposits.

VI SAMPLING PROCEDURES

A Fauna

1 Qualitative sampling determines the variety of species occupying an area. Samples may be taken by any method that will capture representatives of the species present. Collections from such samplings indicate changes in the environment, but generally do not accurately reflect the degree of change. Mayflies, for example, may be reduced from 100 to 1 per square meter. Qualitative data would indicate the presence of both species, but might not necessarily delineate the change in predominance from mayflies to sludgeworms. The stop net or kick sampling technique is often used.

2 Quantitative sampling is performed to observe changes in predominance. The most common quantitative sampling tools are the Petersen, Ekman, and Ponak grabs and the Surber stream bottom or square-foot sampler. Of these, the Petersen grab samples the widest variety of substrates. The Ekman grab is limited to fine-textured and soft substrates, such as silt and sludge, unless hydraulically operated.

The Surber sampler is designed for sampling riffle areas; it requires moving water to transport dislodged organisms into its net and is limited to depths of two feet or less.

Kick samples of one minute duration will usually yield around 1,000 macroinvertebrates per square meter (10.5 X a one minute kick= organisms/m^2).

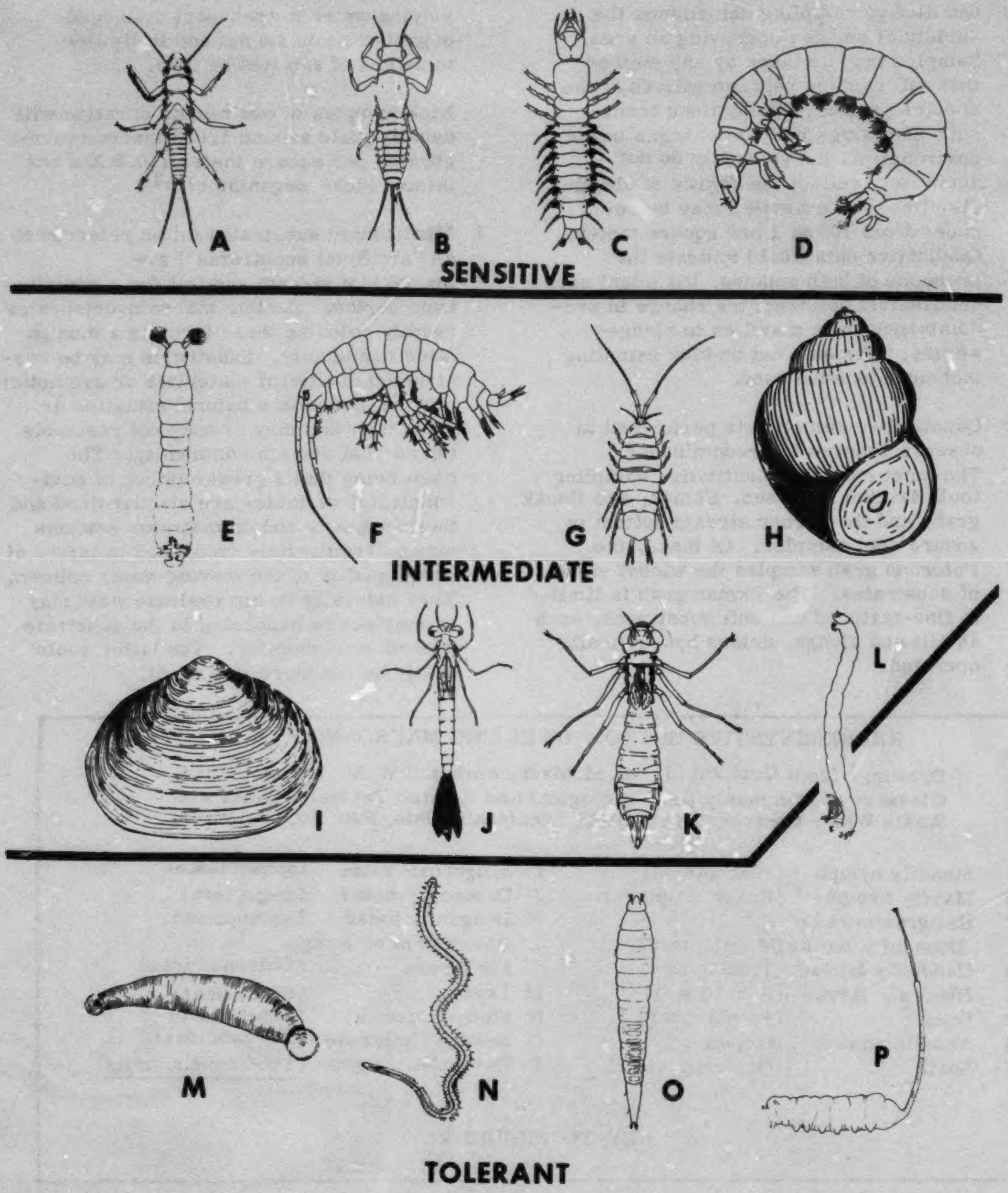
3 Manipulated substrates (often referred to as "artificial substrates") are placed in a stream and left for a specific time period. Benthic macroinvertebrates readily colonize these forming a manipulated community. Substrates may be constructed of natural materials or synthetic; may be placed in a natural situation or unnatural; and may or may not resemble the normal stream community. The point being that a great number of environmental variables are standardized and thus upstream and downstream stations may be legitimately compared in terms of water quality of the moving water column. They naturally do not evaluate what may or may not be happening to the substrate beneath said monitor. The latter could easily be the more important.

REPRESENTATIVE BOTTOM-DWELLING MACROANIMALS

Drawings from Geckler, J., K. M. Mackenthun and W. M. Ingram, 1963. Glossary of Commonly Used Biological and Related Terms in Water and Waste Water Control, DHEW, PHS, Cincinnati, Ohio, Pub. No. 999-WP-2.

A	Stonefly nymph	(Plecoptera)	I	Fingernail clam	(Sphaeriidae)
B	Mayfly nymph	(Ephemeroptera)	J	Damselfly naiad	(Zygoptera)
C	Hellgrammite or Dobsonfly larvae	(Megaloptera)	K	Dragonfly naiad	(Anisoptera)
D	Caddisfly larvae	(Trichoptera)	L	Bloodworm or midge fly larvae	(Chironomidae)
E	Black fly larvae	(Simuliidae)	M	Leech	(Hirudinea)
F	Scud	(Amphipoda)	N	Sludgeworm	(Tubificidae)
G	Aquatic sowbug	(Isopoda)	O	Sewage fly larvae	(Psychodidae)
H	Snail	(Gastropoda)	P	Rat-tailed maggot	(Tubifera-Eristalis)

KEY TO FIGURE 2


A
B
C
D
SENSITIVE
E
F
G
H
INTERMEDIATE
I
J
K
L
M
N
O
P
TOLERANT

4 Invertebrates which are part of the benthos, but under certain conditions become carried downstream in appreciable numbers, are known as Drift.

Groups which have members forming a conspicuous part of the drift include the insect orders Ephemeroptera, Trichoptera, Plecoptera and the crustacean order Amphipoda.

Drift net studies are widely used and have a proven validity in stream water quality studies.

5 The collected sample is screened with a standard sieve to concentrate the organisms; these are sorted from the retained material, and the number of each kind determined. Data are then adjusted to number per unit area, usually to number of bottom organisms per square meter.

6 Independently, neither qualitative not quantitative data suffice for thorough analyses of environmental conditions. A cursory examination to detect damage may be made with either method, but a combination of the two gives a more precise determination. If a choice must be made, quantitative sampling would be best, because it incorporates a partial qualitative sample.

7 Studies have shown that a significant number and variety of macroinvertebrates inhabit the hyporheic zone in streams. As much as 30% of the macroinvertebrates may be below 5 cm in this hyporheic zone. Most samples and sampling techniques do not penetrate the substrate below the 5 cm depth. All quantitative studies must take this and other substrate factors into account when absolute figures are presented on standing crop and numbers per square meter, etc.

B Flora

1 Direct quantitative sampling of naturally growing bottom algae is difficult. It is basically one of collecting algae from a standard or uniform area of the bottom substrates without disturbing the delicate growths and thereby distort the sample. Indirect quantitative sampling is the best available method.

2 Manipulated substrates, such as wood blocks, glass or plexiglass slides, bricks, etc., are placed in a stream. Bottom-attached algae will grow on these artificial substrates. After two or more weeks, the artificial substrates are removed for analysis. Algal growths are scraped from the substrates and the quantity measured. Since the exposed substrate area and exposure periods are equal at all of the sampling sites, differences in the quantity of algae can be related to changes in the quality of water flowing over the substrates.

VII ANALYSES OF MICROFLORA

A Enumeration

1 The quantity of algae on manipulated substrates can be measured in several ways. Microscopic counts of algal cells and dry weight of a algal material are long established methods.

2 Microscopic counts involve thorough scraping, mixing and suspension of the algal cells. From this mixture an aliquot of cells is withdrawn for enumeration under a microscope. Dry weight is determined by drying and weighing the algal sample. then igniting the sample to burn off the algal materials, leaving inert inorganic materials that are again weighed.

The difference between initial dry weight and weight after ignition is attributed to algae.

3 Any organic sediments, however, that settle on the substrate along with the algae are processed also.

Thus, if organic wastes are present appreciable errors may enter into this method.

B Chlorophyll Analysis

1 During the past decade, chlorophyll analysis has become a popular method for estimating algal growth. Chlorophyll is extracted from the algae and is used as an index of the quantity of algae present. The advantages of chlorophyll analysis are rapidity, simplicity, and vivid pictorial results.

2 The algae are scrubbed from the artificial substrate samples, ground, then each sample is steeped in equal volumes, 90% aqueous acetone, which extracts the chlorophyll from the algal cells. The chlorophyll extracts may be compared visually.

3 Because the cholorophyll extracts fade with time, colorimetry should be used for permanent records. For routine records, simple colorimeters will suffice. At very high cholorophyll densities, interference with colorimetry occurs, which must be corrected through serial dilution of the sample or with a nomograph.

C Autotrophic Index

The chlorophyll content of the periphyton is used to estimate the algal biomass and as an indicator of the nutrient content (or trophic Status) or toxicity of the water and the taxonomic composition of the community. Periphyton growing in surface water relatively free of organic pollution consists largely of algae, which contain approximately 1 to 2 percent chlorophyll a by dry weight. If dissolved or particulate organic matter is present in high concentrations, large populations of filamentous bacteria, stalked protozoa, and other nonchlorophyll bearing microorganisms develop and the percentage of chlorophyll is then reduced. If the biomass-chlorophyll a relationship is expressed as a ration (the autotrophic index), values greater than 100 may result from organic pollution (Weber and McFarland, 1969; Weber, 1973).

$$\text{Autotrophic Index} = \frac{\text{Ash-free Wgt (mg/m}^2\text{)}}{\text{Chlorophyll a (mg/m}^2\text{)}}$$

VIII MACROINVERTEBRATE ANALYSES

A Taxonomic

The taxonomic level to which animals are identified depends on the needs, experience, and available resources. However, the taxonomic level to which identifications are carried in each major group should be constant throughout a given study.

B Biomass

Macroinvertebrate biomass (weight of organisms per unit area) is a useful quantitative estimation of standing crop.

C Reporting Units

Data from quantitative samples may be used to obtain:

1 Total standing crop of individuals, or biomass, or both per unit area or unit volume or sample unit, and

2 Numbers of biomass, or both, of individual taxa per unit area or unit volume or sample unit.

3 Data from devices sampling a unit area of bottom will be reported in grams dry weight or ash-free dry weight per square meter (gm/m^2), or numbers of individuals per square meter, or both.

4 Data from multiplate samplers will be reported in terms of the total surface area of the plates in grams dry weight or ash-free dry weight or numbers of individuals per square meter, or both.

5 Data from rock-filled basket samplers will be reported as grams dry weight or numbers of individuals per sampler, or both.

IX FACTORS INVOLVED IN DATA INTERPRETATION

Two very important factors in data evaluation are a thorough knowledge of conditions under which the data were collected and a critical assessment of the reliability of the data's representation of the situation.

A Maximum-Minimum Values

The evaluation of physical and chemical data to determine their effects on aquatic organisms is primarily dependent on maximum and minimum observed values. The mean is useful only when the data are relatively uniform. The minimum or maximum values usually create acute conditions in the environment.

B Identification

Precise identification of organisms to species requires a specialist in limited taxonomic groups. Many immature aquatic forms have not been associated with the adult species. Therefore, one who is certain of the genus but not the species should utilize the generic name, not a potentially incorrect species name. The method of interpreting biological data on the basis of numbers of kinds and numbers of organisms will typically suffice.

C Lake and Stream Influence

Physical characteristics of a body of water also affect animal populations. Lakes or impounded bodies of water support different faunal associations from rivers. The number of kinds present in a lake may be less than that found in a stream because of a more uniform habitat. A lake is all pool, but a river is composed of both pools and riffles. The nonflowing water of lake exhibits a more complete settling of particulate organic matter that naturally supports a higher population of detritus consumers. For these reasons, the bottom fauna of a lake or impoundment, or stream pool cannot be directly compared with that of a flowing stream riffle.

D Extrapolation

How can bottom-dwelling macrofauna data be extrapolated to other environmental components? It must be borne in mind that a component of the total environment is being sampled. If the sampled component exhibits changes, then so must the other interdependent components of the environment. For example, a clean stream with a wide variety of desirable bottom organisms would be expected to have a wide variety of desirable bottom fishes; when pollution reduces the number of bottom organisms, a comparable reduction would be expected in the number of fishes. Moreover, it would be logical to conclude that any factor that eliminates all bottom organisms would eliminate most other aquatic forms of life. A clean stream with a wide variety of desirable bottom organisms would be expected to permit a variety of recreational, municipal and industrial uses.

E Expression of Data

1 Standing crop and taxonomic composition

Standing crop and numbers of taxa (types or kinds) in a community are highly sensitive to environmental perturbations resulting from the introduction of contaminants. These parameters, particularly standing crop, may vary considerably in unpolluted habitats, where they may range from the typically high standing crop of littoral zones of glacial lakes to the sparse fauna of torrential soft-water streams. Thus, it is important that comparisons are made only between truly comparable environments.

2 Diversity

Diversity indices are an additional tool for measuring the quality of the environment and the effect of induced stress on the structure of a community of macroinvertebrates. Their use is based on the generally observed phenomenon that relatively undisturbed environments support communities having large numbers of species with no individual species present in overwhelming abundance. If the species in such a community are ranked on the basis of their numerical abundance, there will be relatively few species with large numbers of individuals and large numbers of species represented by only a few individuals. Many forms of stress tend to reduce diversity by making the environment unsuitable for some species or by giving other species a competitive advantage.

3 Indicator-organism scheme (rat-tailed maggot studies)

a For this technique, the individual taxa are classified on the basis of their tolerance or intolerance to various levels of putrescible wastes. Taxa are classified according to their presence or absence of different environments as determined by field studies. Some reduce data based on the presence or absence of indicator organisms to a simple numerical form for ease in presentation.

b "Biologists are engaging in fruitless exercise if they intend to make any decisions about indicator organisms by operating at the generic level of macroinvertebrate identifications." (Resh and Unzicker)

4 Reference station methods

Comparative or control station methods compare the qualitative characteristics of the fauna in clean water habitats with those of fauna in habitats subject to stress. Stations are compared on the basis of richness of species.

If adequate background data are available to an experienced investigator, these techniques can prove quite useful—particularly for the purpose of demonstrating the effects of gross to moderate organic contamination on the macroinvertebrate community. To detect more subtle changes in the macroinvertebrate community, collect quantitative data on numbers or biomass of organisms. Data on the presence of tolerant and intolerant taxa and richness of species may be effectively summarized for evaluation and presentation by means of line graphs, bar graphs, pie diagrams, histograms, or pictoral diagrams.

X IMPORTANT ASSOCIATED ANALYSES

A The Chemical Environment

1 Dissolved oxygen

2 Nutrients

3 Toxic materials

4 Acidity and alkalinity

5 Etc.

B The Physical Environment

1 Suspended solids

2 Temperature

3 Light penetration

4 Sediment composition

5 Etc.

XI AREAS IN WHICH BENTHIC STUDIES CAN BEST BE APPLIED

A Damage Assessment or Stream Health

If a stream is suffering from abuse the biota will so indicate. A biologist can determine damages by looking at the "critter" assemblage in a matter of minutes. Usually, if damages are not found, it will not be necessary to alert the remainder of the agency's staff, pack all the equipment, pay travel and per diem, and then wait five days before enough data can be assembled to begin evaluation.

B By determining what damages have been done, the potential cause "list" can be reduced to a few items for emphasis and the entire "wonderful worlds" of science and engineering need not be practiced with the result that much data are discarded later because they were not applicable to the problem being investigated.

C Good benthic data associated with chemical, physical, and engineering data can be used to predict the direction of future changes and to estimate the amount of pollutants that need to be removed from the waterways to make them productive and useful once more.

D The benthic macroinvertebrates are an easily used index to stream health that citizens may use in stream improvement programs. "Adopt-a-stream" efforts have successfully used simple macroinvertebrate indices.

E The potential for restoring biological integrity in our flowing streams using macroinvertebrates has barely been touched.

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Descriptors: Aquatic Life, Benthos, Water Quality, Degradation, Environmental Effects, Trophic Level, Biological Communities, Ecological Distributions

STATISTICS AS AN ECOLOGICAL TOOL

I SAMPLING

A Introduction

The sampling problem is seldom fully appreciated in spite of the fact that the ecologist relies heavily on sampling experiments as a source of information for decisions.

1 Conditions necessary

Two main considerations should be satisfied:

a Avoid bias

Bias often arises from particular experimental conditions which almost never represent the exact model of the situation about which inferences are desired.

b The sample should yield information on its own accuracy.

To obtain a valid estimate of sampling error, each batch of material must be so sampled that two or more sampling units are obtained from it.

2 Advantages over complete enumeration.

These include: reduced cost, greater speed, greater scope, greater accuracy.

B Definitions

We refer to sampling from finite populations. These are populations from which the experimental units can be enumerated, and consequently, randomly sampled. In sampling finite populations, there are several ways in which a selection may be made. These include:

1 Simple random sampling

The population is listed and the plan and sample size fixed. The important criterion is that each possible sample has the same probability of being selected. In the actual process random number tables are used, and the sampling units are drawn independently.

2 Systematic sampling

In systematic sampling the samples are equally spaced throughout the area or population to be sampled. There are analytic difficulties connected with this procedure.

3 Stratified random sampling

The material or area is divided into uniform groups or strata and a number of observations are taken from each stratum.

Figure 1 illustrates random, systematic and stratified random sampling along a line.

C Simple Random Sampling - Notation

Generally, guides for using simple random sampling are: a) when the population is not highly variable, b) when sampling for proportions, use simple random sampling when the true value lies between 20 and 80%.

1 Let Y_i be the i th observation in the population.

Population size: N
Sample size: n
Population mean:

$$\bar{Y} = \frac{\Sigma_i Y_i}{N} = \frac{Y}{N}, \text{ for a continuous variable}$$

$$P = \frac{A}{N}, \text{ for a proportion}$$

For a proportion, $Y_i = 0$ or 1. Replace $\Sigma_i Y_i$ by A.

Sample mean:

$$\hat{Y} = \bar{y} = \frac{\Sigma_i Y_i}{n} = \frac{y}{n}$$, for a continuous variable

$$P = P = \frac{a}{n}$$, for a proportion

For a proportion, replace ΣY_i by a.

Population variance:

$$S^2 = \frac{\Sigma_i (Y_i - \bar{\bar{Y}})^2}{N - 1}$$

Population variance for a mean:

$$S_{\bar{y}}^2 = \frac{S^2}{n} \left(\frac{N - n}{N}\right)$$

$$S_p^2 = \frac{PQ}{n} \left(\frac{N - n}{N - 1}\right) \text{ where } Q = 1 - P$$

Sample variance:

$$s^2 = \frac{\Sigma_i (Y_i - \bar{y})^2}{n - 1}$$ an unbiased estimate or S^2

For computational purposes:

$$s^2 = \frac{\Sigma Y_i^2 - (\Sigma Y_i)^2 / n}{n - 1}$$

Sample variance of a mean:

$$s_{\bar{y}}^2 = \frac{s^2}{n} \left(\frac{N - n}{N}\right)$$

$$s_p^2 = \frac{pq}{n - 1} \; \frac{N - n}{N} \quad \text{when } q = 1 - p$$

(N - n)/N is the finite population correlation factor or Fpc. It is also written as 1 - n/N and n/N is the sampling fraction. If it is small, say 5%, it is usually neglected. The confidence interval for a mean:

$$CI = \bar{y} \pm t \left[\frac{s}{\sqrt{n}} \sqrt{\frac{N - n}{N}} \right]$$

An approximation to the confidence interval for a proportion:

$$CI = p \pm t \sqrt{\frac{pq}{n} \; \frac{N - n}{N - 1}}$$

Note: In the formula above, estimated standard deviation is comparible to population quantity, not sample quantity. Above more commonly used.

Example: Attribute sampling. Of 1000 lobsters, 400 are sampled and 120 harbor a certain parasite. The estimated proportion with the parasite is a/n = 120/400 = 0.30. The 95% confidence interval is .30 ± .048 using the formula for CI above with $t_{.05, \infty} = 1.96$.

Example: Continuous variable. The following 11 measurements represent a random sample from a set of 50 objects. Their measurements are: 3, 6, 6, 12, 9, 12, 10, 9, 16, 14, 17. The mean is 10 and the standard deviation is 4. The 95% confidence interval for the mean is 10 ± 2.34, $t_{.05, 10}$ is 2.228.

D Stratified Sampling. Notation.

To increase precision we may increase n or reduce population variance. A good way to decrease population variance is to construct relatively homogeneous strata from the sampling units. Variations in strata means in the population do not contribute to the sampling error of the estimate of the population mean.

Let Y_{ki} be the ith observation in the kth stratum, k = 1, ... , s. Strata sizes, means and variances are described by N_k, $\bar{Y}_{k_2}$, or P_k, and S_k^2 with corresponding sample values of n_k, $\bar{y}_k$ or p_k and s_k.

Stratum mean and variance:

$$\hat{\bar{Y}}_k = \frac{\sum_{i=1}^{N_k} Y_{ki}}{N_k} = \frac{Y_k}{N_k}$$

$$P_k = \frac{A_k}{N_k}$$

$$S_k^2 = \frac{\sum_{i=1}^{N_k} (Y_{ki} - \bar{Y}_k)^2}{N_k - 1}$$

Sample mean and variance for kth stratum:

$$\hat{\bar{Y}}_k = \bar{y}_k = \frac{\sum_{i-1}^{n_k} Y_{ki}}{n_k} = \frac{y_k}{n_k}$$

$$\hat{P}_k = p_k = \frac{a_k}{n_k}$$

$$s_k^2 = \sum_{i=1}^{n_k} \frac{(Y_{ki} - \bar{y}_k)^2}{n_k - 1}$$

Let $N = \sum_k N_k$ and $n = \sum_k n_k$ and $N_k/N = W_k$

where W stands for weight.

Population mean (st mean stratified):

$$\bar{Y}_{st} = \frac{\sum_k N_k \bar{Y}_k}{N} = \sum_k W_k \bar{Y}_k$$

$$P_{st} = \sum_k \frac{N_k P_k}{N} = \sum_k W_k \bar{P}_k$$

Estimate of population mean:

$$\hat{\bar{Y}}_{st} = \bar{y}_{st} = \sum_k \frac{N_k \bar{y}_k}{N} = \sum_k W_k \bar{y}_k$$

$$\hat{P}_{st} = p_{st} = \frac{\sum_k N_k p_k}{N} = \sum_k W_k p_k$$

(sample means are $\bar{y} = \Sigma n_k \bar{y}_k / n$ and

$P = \sum_k n_k p_k / n$.)

Variance of estimate of population mean:

$$\sigma^2(\bar{y}_{st}) = \sum_k \frac{N_k}{N} \frac{S_k^2}{n_k} \frac{N_w - n_k}{N_k}$$

$$= \frac{1}{N^2} \Sigma N_k (N_k - n_k) \frac{S_k^2}{n_k}$$

$$\sigma^2(p_{st}) = \sum_k \frac{N_w^2}{N^2} \frac{P_k Q_k}{n_k} \frac{N_k - n_k}{N_k - 2}$$

Sample variance of the estimate of the population mean:

$$s^2(\bar{y}_{st}) = \frac{1}{N^2} \sum_k N_k (N_k - n_k) \frac{s_k^2}{n_k}$$

$$= \sum_k W_k^2 \frac{s_k^2}{n_k} - \frac{1}{N} \sum_k W_k s_k^2$$

In formulas where pfc occurs it is ignored if small when computing confidence intervals.

Note: Stratified sampling may be used with proportional allocation or with a variable sampling fraction.

II EXPERIMENTATION

A Planning Steps

1 Decide and define what the experiment is intended to do. Specify population to which results are intended to apply.

2 Gauge the probable accuracy of the results likely to be obtained.

This is usually done by:

a Estimating the coefficient of variation, the precentage variation in the observations that cannot be accounted for by experimental factors.

b Specifying a value for the accuracy desired in the treatment effect, expressed as a percentage of the overall mean.

c Specifying the probability for the true value of the difference to fall within assigned limits.

B Experimental Design

Definition includes:

1 the set of treatments selected for comparison,

2 the specification of the units (animals, plants, plots, samples) to which the treatments are to be applied,

3 the rules by which the treatments are allocated to experimental units,

4 specification of measurements or other records to be made on each unit.

C Choosing the design includes deciding:

1 whether the design is unifactor or factorial - unifactor implies one treatment (factor) to be tested holding others constant. Factorial implies all combinations of the different treatments or factors.

2 whether to group observations

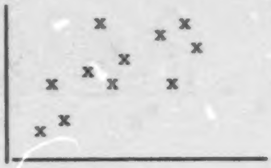
3 whether the number of treatments or conclusions is too large to allow full replication - i. e. . com
incomplete bl

D Classification of
signs are shown

III DATA INTERPRE

A Associated Measurements

Ecologists frequently obtain data which may look something like that shown below, and wish to perform statistical tests with these data.



B Procedure

1 Guess the nature of the relationships from the graph. That is: we may have

$$y = bx$$

$$y = a + bx$$

$$y = ax^{b} \text{ etc.}$$

2 Test the guess by graphical inspection.

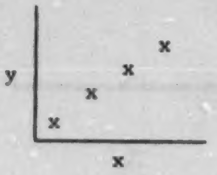
3 Perform the appropriate computations on the transformed data, and make the appropriate statistical tests. Any equation which can be converted to the form:

$$y = a + bx$$

can be handled by ordinary regression computing techniques, described in any standard statistical text.

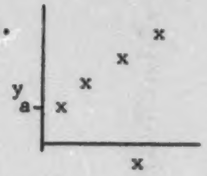
C Examples of Linear Relationships

1.



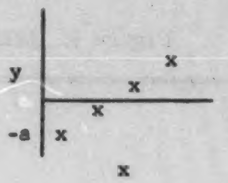
At $x = 0$, $y = 0$. Thus, there is no intercept, and $y = bx$

2.

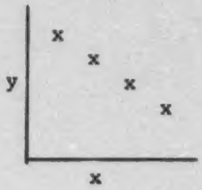


At $x = 0$, $y = a$. There is an intercept. $y = a + bx$

3.



At $x = 0$, $y = -a$. Thus $y = -a+bx$



Negative slope, thus $y = a - bx$

D Other graphs, postulated relations, and graphical tests include the following:

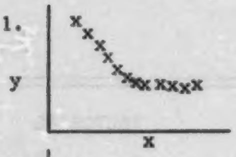
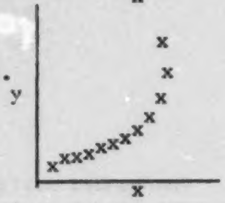
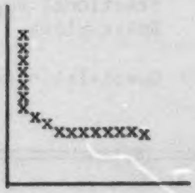
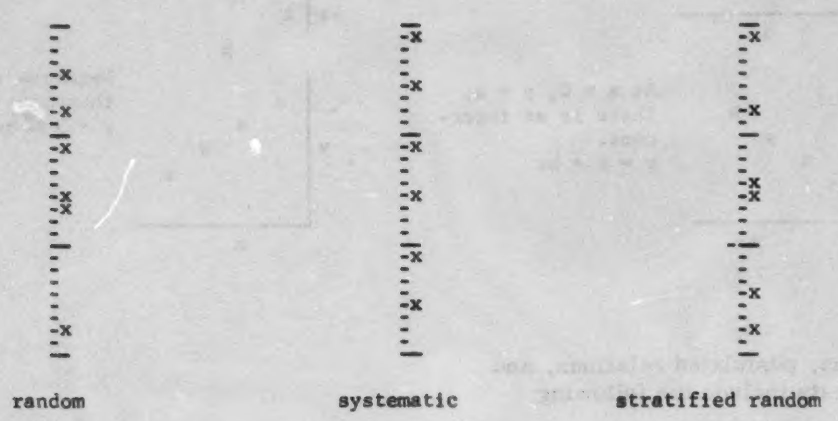
Raw Data	Possible Equation	Test giving linearity if equation describes data
1. 	$y = ac^{-bx}$	$\log y = \log a - bx$ plot y against x on semi log paper
2. 	$y = ax^{b}$	$\log y = \log a + b \log x$ Plot y against x on log-log paper
3. 	$y = \frac{1}{bx}$	Plot y against $\frac{1}{x}$

Figure 1. METHODS OF SAMPLING



random systematic stratified random

Figure 2

		UNIFACTOR	FACTORIAL
COMPLETE BLOCK	one grouping	random blocks	
	two groupings	Latin squares	
INCOMPLETE BLOCK	one grouping	Balanced incomplete blocks Lattice designs	Confounded designs Fractional replications Split-plots
	two groupings	Lattice squares Youden squares	Quasi-latin squares

E Calculations in Regression and Correlation

1 The basic observations are in pairs of associated observations represented by (x, y). We assume x and y follow, at least approximately, a bivariate normal distribution.

2 A convenient desk calculator procedure is to find the sums, and sums of squares of products as follows:

$$(1) \quad \sum^{n} X \qquad \sum Y$$

$$\sum X^2 \qquad \Sigma\text{[illegible]} \qquad \sum XY$$

Next, calculate the three quantities in the line below:

$$(2) \quad \frac{1}{n}(\Sigma X)^2, \quad \frac{1}{n}(\Sigma Y)^2, \quad \frac{1}{n}(\Sigma X)(\Sigma Y)$$

Subtract each of these from the last line of set (1) to give the corrected sums of squares and cross-products.

$$(3) \quad \Sigma x^2 = \Sigma X^2 - \frac{1}{n}(\Sigma X)^2, \quad \Sigma y^2 = \Sigma Y^2 - \frac{1}{n}(\Sigma Y)^2,$$

$$\Sigma xy = \Sigma XY - \frac{1}{n}(\Sigma X)(\Sigma Y)$$

The estimate of the true regression coefficient is given by:

$$(4) \quad b = \frac{\Sigma xy}{x^2}$$

and the constant a is given by

$a = \bar{y} - b\bar{x}$.

The variance of the deviations of y from the regression line is estimated by:

$$s^2 = \frac{1}{n-2}\left(\Sigma y^2 - \frac{\Sigma xy^2}{\Sigma x^2}\right)$$

The correlation coefficient r is calculated as:

$$r = \frac{\Sigma xy}{\sqrt{(\Sigma x^2)(\Sigma y^2)}}$$

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Descriptors: Statistics, Statistical Methods, Data Processing and Ecology

THE INTERPRETATION OF BIOLOGICAL DATA WITH REFERENCE TO WATER QUALITY

I INTRODUCTION

Sanitary engineers like to have data presented to them in a readily assimilable form and some of them seem a little impatient with biologists who appear unable to provide definite quantitative criteria applicable to all kinds of water conditions. I think the feeling tends to be that this is the fault of biologists, and if they would only pull themselves out of the scientific stone-age all would be well. I will try to explain here why I believe that biological data can never be absolute nor interpretable without a certain amount of expertise. In this respect biologists resemble medical men who make their diagnoses against a complex background of detailed knowledge. Anyone can diagnose an open wound but it takes a doctor to identify an obscure disease; and although he can explain how he does it he cannot pass on his knowledge in that one explanation. Similarly, one does not need an expert to recognize gross organic pollution, but only a biologist can interpret more subtle biological conditions in a water body; and here again he can explain how he does it, but that does not make his hearer a biologist. Beck (1957) said something similar at a previous symposium in Cincinnati in 1956.

II THE COMPLEXITY OF BIOLOGICAL REACTIONS TO WATER CONDITIONS

A Complexity of the Aquatic Habitat

The aquatic habitat is complex and consists not only of water but of the substrata beneath it, which may be only indirectly influenced by the quality of the water. Moreover, in biological terms, water quality includes such features as rate of flow and temperature regime, which are not considered of direct importance by the chemist.

To many animals and plants, maximum summer temperature or maximum rate of flow is just as important as minimum oxygen tension. The result is that inland waters provide an enormous array of different combinations of conditions, each of which has its own community of plants and animals; and the variety of species involved is very great. Thus, for example, Germany has about 6000 species of aquatic animals (Illies 1961a) and probably at least as many species of plants. Yet Europe has a rather restricted fauna because of the Pleistocene ice age; in most other parts of the world the flora and fauna are even richer.

B Distribution of Species and Environmental Factors

We know something about the way in which species are distributed in the various habitats, especially in the relatively much studied continent of Europe, but we have, as yet, little idea as to what factors or combination of factors actually control the individual species.

1 Important ecological factors

Thus, it is possible to list the groups of organisms that occur in swift stony upland rivers (rhithron in the sense of Illies, 1961b) and to contrast them with those of the lower sluggish reaches (potamon). Similarly we know, more or less, the different floras and faunas we can expect in infertile (oligotrophic) and fertile (eutrophic) lakes. We are, however, much less informed as to just what ecological factors cause these differences. We know they include temperature and its yearly amplitude; oxygen, particularly at minimal levels; plant nutrients, such as nitrate, phosphate, silica, and bicarbonate; other ions in solution, including calcium, chloride, and possibly hydrogen; dissolved organic matter, which is necessary for some bacteria and fungi and probably for some algae; the nature of the substratum; and current.

2 Complexity of interacting factors

We also know these factors can interact in a complex manner and that their action on any particular organism can be indirect through other members of the biota.

a Induced periphyton growths

Heavy growths of encrusting algae induced by large amounts of plant nutrients, or of bacteria induced by ample supplies of organic matter, can eliminate or decimate populations of lithophile insects by simple mechanical interference. But the change does not stop there: the growths themselves provide habitats for the animals, such as Chironomidae and Naidid worms, which could not otherwise live on the stones.

b Oxygen levels and depositing substrates

If oxygen conditions over a muddy bottom reach levels just low enough to be intolerable to leeches, tubificid worms, which the leeches normally hold in check, are able to build up to enormous numbers especially as some of their competitors (e.g. Chironomus) are also eliminated.

c Oxygen levels and non muddy substrates

One then finds the typical outburst of sludge worms, so often cited as indicators of pollution. This does not happen if the same oxygen tension occurs over sand or rock, however, as these are not suitable substrata for the worms. Many such examples could be given, but they would only be ones we understand: there must be a far greater number about which we know nothing.

d One must conclude, therefore, that quite simple chemical changes can produce far-reaching biological effects; that we only understand a small proportion of them; and that they are not always the same.

3 Classic examples

This seems like a note of despair, however, if water quality deviates too far from normal, the effects are immediately apparent. Thus, poisonous substances eliminate many species and may leave no animals (Hynes 1960); excessive quantities of salt remove all leeches, amphipods, and most insects and leave a fauna consisting largely of Chironomidae, caddis worms, and oligochaetes (Albrecht 1954) and excessive amounts of dissolved organic matter give rise to carpets of sewage fungus, which never occur naturally. Here no great biological expertise is needed, and there is little difficulty in the communication of results. It is when effects are slighter and more subtle that biological findings become difficult to transmit intelligibly to other disciplines.

III THE PROBLEMS IN PRESENTATION OF BIOLOGICAL RESULTS

Because of these difficulties various attempts have been made to simplify the presentation of biological findings, but to my mind none of them is very successful because of the complexity of the subject. Early attempts at systematization developed almost independently on the two sides of the Atlantic, although they had some similarities.

A Early Studies in the United States (Richardson and the Illinois River)

In America, there was a simple division into zones of pollution, e.g. degradation, septic, and recovery, which were characterized in broad general terms. This simple, textbook approach is summarized by Whipple et al. (1947), and serves fairly well for categorizing gross organic pollution such as has been mentioned above. It was, however, soon found by Richardson (1929) during his classical studies on the Illinois River that typical "indicators" of foul conditions, such as Tubificidae and Chironomus, were not always present where they would be expected to occur. This was an early indication that it is not the water quality itself that provides suitable conditions for "pollution faunas," but other, usually associated, conditions - in this instance deposits of rich organic mud. Such conditions may, in fact, be present in places where water quality in no way resembles pollution, e.g., upstream of weirs in trout streams where autumn leaves accumulate and decay and cause the development of biota typical of organically polluted water. Samples must therefore be judged against a background of biological knowledge. Richardson was fully aware of this and was in no doubt about the condition of the Illinois River even in places where his samples showed few or no pollution indicators.

B The European Saprobic System

In Europe, the initial stress was primarily on microorganisms and results were first codified in the early years of the century by Kolkwitz and Marsson. In this "Saprobiensystem," zones of organic pollution similar to those described by the American workers were defined and organisms were listed as characteristic of one or more zones;

TABLE 1

SAPROBIENSYSTEM - A European system of classifying organisms according to their response to the organic pollution in slow moving streams. (22)

Alpha-Mesosaprobic Zone - Area of active decomposition, partly aerobic, partly anaerobic, in a stream heavily polluted with organic wastes.

Beta-Mesosaprobic Zone - That reach of stream that is moderately polluted with organic wastes.

Oligosaprobic Zone - That reach of a stream that is slightly polluted with organic wastes and contains the mineralized products of self-purification from organic pollution, but with none of the organic pollutants remaining.

Polysaprobic Zone - That area of a grossly polluted stream which contains the complex organic wastes that are decomposing primarily by anaerobic processes.

A recent exposition of this list is given by Kolkwitz (1950). It was then claimed that with a list of the species occurring at a particular point it was possible to allocate it to a saprobic zone. This system early met with criticism for several reasons. First,

TABLE 2

SAPROBICITY LEVELS ACCORDING TO THE TROPHIC STRUCTURE OF THE COMMUNITIES OF ORGANISMS

Saprobicity Level	Structure of the Communities of Organisms
I β-oligosaprobic	Balanced relationship between producers, consumers and destroyers; the communities of organisms are poor in individuals but there is a moderate variety of species, small biomass and low bioactivity.
II α-oligosaprobic	Balanced relationship between producers, consumers and destroyers; communities of organisms are rich in individuals and species with a large biomass and high bioactivity.
III β-mesosaprobic	Substantially balanced relationship between producers, consumers and destroyers; a relative increase in the abundance of destroyers and, accordingly, of the consumers living off them; communities of organisms are rich in individuals and species with a large biomass and high bioactivity.
IV α-mesosaprobic	Producers decline as compared with an increase in consumers and destroyers; mixotrophic and amphitrophic forms predominate among the producers; communities of organisms rich in individuals but poor in species with a large biomass and extremely high bioactivity; still only few species of macro-organisms; mass development of bacteria and bacteria-eating ciliates.
V β-polysaprobic	Producers drastically decline; communities of organisms are extremely rich in individuals but poor in species with a large biomass and high bioactivity; macrofauna represented only by a few species of tubificids and chironomids; as in IV these are in great abundance; mass development of bacteria and bacteria-eating ciliates.
VI α-polysaprobic	Producers are absent; the total biomass is formed practically solely by anaerobic bacteria and fungi; macro-organisms are absent; flagellates outnumber ciliates amongst the protozoa.

Saprobicity - "Within the bioactivity of a body of water, Saprobicity is the sum total of all those metabolic processes which are the antithesis of primary production. It is therefore the sum total of all those processes which are accompanied by a loss of potential energy." Part I, Prague Convention.

all the organisms listed occurred in natural habitats--they were not evolved in polluted water--and there was much doubt as to the placing of many of the species in the lists. The system, however, did serve to codify ecological knowledge about a long list of species along an extended trophic scale. Its weaknesses appeared to be merely due to lack of knowledge; such a rigid system took far too little account of the complexity of the reaction of organisms to their habitats. For instance, many organisms can be found, albeit rarely, in a wide range of conditions and others may occur in restricted zones for reasons that have nothing to do with water quality. We often do not know if organisms confined to clean headwaters are kept there by high oxygen content, low summer temperatures, or inability to compete with other species under other conditions. In the swift waters of Switzerland the system broke down in that some organisms appeared in more polluted zones than their position in the lists would indicate. Presumably here the controlling factor was oxygen, which was relatively plentiful in turbulent cold water. In a recent series of experiments, Zimmerman (1962) has proven that current alone has a great influence on the biota, and identically polluted water flowing at different speeds produces biotic communities characteristic of different saprobic levels. He finds this surprising, but to me it seems an expected result, for the reasons given above.

C Recent Advances in the Saprobic System

1 Perhaps Zimmerman's surprise reflects the deeply rooted entrenchment of the Saprobiensystem in Central Europe. Despite its obvious shortcomings it has been revised and extended. Liebmann (1951) introduced the concept of considering number as well as occurrence and very rightly pointed out that the community of organisms is what matters rather than mere species lists. But he did not stress the importance of extrinsic factors, such as current, nor that the system can only apply to organic pollution and that different types of organic pollution differ in their effects; e.g., carbohydrate solutions from paper works produce different results from those of sewage, as they contain little nitrogen and very different suspended solids. Other workers (Sladecek 1961 and references therein) have subdivided the more polluted zones, which now, instead of being merely descriptive, are considered to represent definite ranges of oxygen content, BOD, sulfide, and even *E. coli* populations. Every water chemist knows that BOD and oxygen content are not directly related and to assume that either should be more than vaguely related to the complexities of biological reactions seems to me to indicate a fundamental lack of ecological understanding. I also think it is damaging to the hope of mutual understanding between the various disciplines concerned with water quality to give the impression that one can expect to find a close and rigid relationship between water quality measurements as assessed by different sets of parameters. Inevitably these relationships vary with local conditions; what applies in a sluggish river in summer will certainly not apply to a mountain stream or even to the same river in the winter. Correlation of data, even within one discipline, needs understanding, knowledge, and judgment.

2 Caspers and Schulz (1960) showed that the failure of the system to distinguish between waters that are naturally productive and those artifically enriched can lead to absurd results. They studied a canal in Hamburg, which because of its urban situation can only be regarded as grossly polluted. Yet it develops a rich plankton,

the composition of which, according to the system, shows it to be virtually clean.

D Numerical Application of the Saprobic System

Once the Saprobiensystem was accepted it was logical to attempt to reduce its findings to simple figures or graphs for presentation of results. Several such methods were developed, which are described by Tumpling (1960), who also gives the original references. In all these methods, the abundance of each species is recorded on some sort of logarithmic scale (e.g. 1 for present, 3 for frequent, 5 for common, etc.). The sums of these abundances in each saprobic level are plotted on graphs, the two most polluted zones showing as negative and others as positive. Or, the various saprobic levels are given numerical values [1 for oligosaprobic (clean), 2 for β-mesosaprobic, etc.] and the rating for each species is multiplied by its abundance number. The sum of all these products divided by the sum of all the frequencies gives a "saprobic index" for the locality. Clearly the higher this number, the worse the water quality in terms of organic pollution. In a similar way the so-called "relative Belastung" (relative load) is calculated by expressing the sums of all the abundances of organisms characteristic of the two most-polluted zones as a percentage of the sum of all abundances. Then 100 percent is completely polluted water, and clean localities will give a low number.

E Weaknesses of the Saprobic System

There are various elaborations of these methods, such as sharing of species between zones and taking account of changes in base-line as one passes downstream. None of them, however, eliminates the basic weaknesses of the system nor the fact that, as Caspers and Schulz (1960) point out, there is little agreement between the various authors in the assignment of species to the different levels. Therefore, one gains a number or a figure that looks precise and is easily understood, but it is based on very dubious foundations.

F Comparative North American Systems

Similar systems are indigenous to North America, but were independently evolved.

1 Wurtz (1955) and Wurtz and Dolan (1960) describe a system whereby animals are divided into sensitive-to-pollution and non-sensitive (others are ignored), and also into burrowing, sessile, and foraging species (six classes).

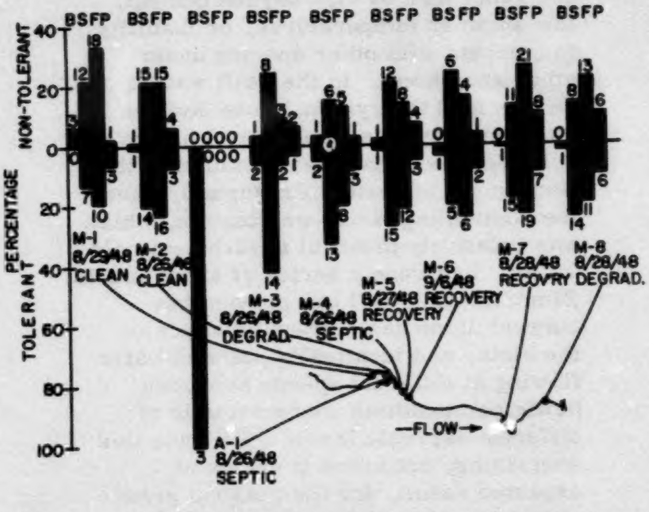

Figure 1. Histograms, based on selected organisms, illustrating stream reaches of clean, degradation, septic, and recovery conditions [after Wurtz] (22)

Numbers of these species represented are plotted for each station as six histograms on the basis of percentage of total number of species. If the constitution of the fauna from control stations or from similar localities is known, it is possible to express numerically "biological depression" (i.e., percentage reduction in total number of species), "biological distortion" (changes in proportions of tolerant and non-tolerant species), and "biological skewness" (changes in the ratios of the three habitat classes). Such results must, of course, be evaluated, and the definition of tolerance is quite subjective; but the method has the advantages of simplicity and dependence on control data. Like the Saprobiensystem, however, it can have no universal validity. It also suffers from the fact that it takes no account of numbers; a single specimen, which may be there by accident, carries as much weight as a dense population.

2 Patrick (1949) developed a similar system in which several clean stations on the water body being investigated are chosen, and the average number of species is determined occurring in each of seven groups of taxa chosen because of their supposed reaction to pollution. These are then plotted as seven columns of equal height, and data from other stations are plotted on the same scale; it is assumed that stations differing markedly from the controls will show biological imbalance in that the columns will be of very unequal heights. Number is indicated by double width in any column containing species with an unusual number of individuals.

I have already questioned the usefulness of this method of presentation (Hynes 1960), and doubt whether it gives any more readily assimilable data than simple tabulation; it does however, introduce the concept of ecological imbalance.

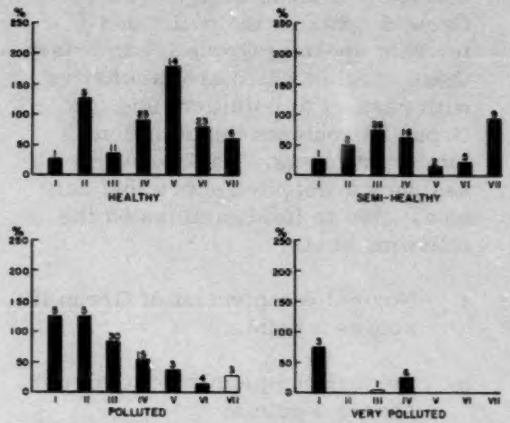

Figure 2. Histograms, based on selected organisms, illustrating healthy, semi-healthy, polluted, and very polluted stations in Conestoga Basin, Pa. [after Patrick] (22)

TABLE 3 —**Classification of Groups of Organisms Shown in Figure 2**

GROUP	ORGANISM
I	Blue-green algae; green algae of the genera *Stigeoclonium*, *Spirogyra*, and *Tribonema*; the bdelloid rotifers plus *Cephalodella megalocephala* and *Proales decipiens*
II	Oligochaetes, leeches, and pulmonate snails
III	Protozoa
IV	Diatoms, red algae, and "most of the green algae"
V	All rotifers not included in Group I, clams, gill-breathing snails, and tricladid flatworms
VI	All insects and crustacea
VII	All fish

3 Beak (1964), another author, recognized the need for a concise expression of pollution based on biological information. Toward this end, he developed a method of biological scoring which is based on the frequency of occurrence of certain macroscopic invertebrates obtained from 6 years of study on one river. It will be noted that the Biological Score is a modification and expansion of Beck's Biotic Index.

The indicator organisms are divided into three categories: Group I contains the pollution-tolerant species; Group II comprises those species which are facultative with respect to pollution; and Group III contains the pollution-intolerant forms. Each group is assigned a weighted score that can be allotted to field samples on the following basis:

a Normal complement of Group III scores 3 points.

b Normal complement of Group II scores 2 points.

c Normal complement of Group I scores 1 point.

The scores are additive; thus an unpolluted stream will have a Biological Score of 6. If only pollution-tolerant forms are found, the score will be 1. If no organisms are found, the score will be zero. Furthermore, a score of, 1 or 2 points could be allotted to Group III when less than the normal complement is present. Group II could be treated in a similar manner. This scoring device correlated well with the biological oxygen demand, dissolved oxygen, and solids content of the receiving water. Beak also related his scoring device to the fisheries potential. This relationship is shown in Table 4]

TABLE 4

TENTATIVE RELATIONSHIP OF THE BIOLOGICAL SCORE TO THE FISHERIES POTENTIAL (after Beak, 1964) (30)

Pollution status	Biotic index	Fisheries potential
Unpolluted	6	All normal fisheries for type of water well developed
Slight to moderate pollution	5 or 4	Most sensitive fish species reduced in numbers or missing
Moderate pollution	3	Only coarse fisheries maintained
Moderate to heavy pollution	2	If fish present, only those with high toleration of pollution
Heavy pollution	1	Very little, if any, fishery
Severe pollution, usually toxic	0	No fish

4 It has long been known that ecologically severe habitats contain fewer species than normal habitats and that the few species that can survive the severe conditions are often very abundant as they lack competitors. Examples of this are the countless millions of Artemia and Ephydra in saline lakes and the Tubifex tubifex in foul mud. This idea has often been expressed in terms of diversity, which is some measure of numbers of species divided by number of specimens collected. Clearly, such a parameter is larger the greater the diversity, and hence the normality of the habitat. Unfortunately, though as the number of species in any habitat is fixed, it also decreases as sample size increases so no index of diversity has any absolute value (Hairston 1959). If a definite sample size is fixed, however, in respect to numbers of organisms identified, it is possible to arrive at a constant index.

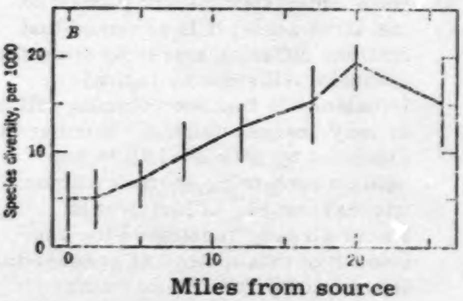

Figure 3. Zooplankton species diversity per thousand individuals encountered in marine systems affected by waste waters from petrochemical industrial wastes. The vertical lines indicate seasonal variations. (30)

5 Patrick et al. (1954) in effect used this concept in a study of diatom species growing on slides suspended in water for fixed periods. They identified 8000 specimens per sample and plotted the results as number of species per interval against number of specimens per species on a logarithmic scale. This method of plotting gives a truncated normal curve for a wide variety of biotic communities. In an ordinarily diverse habitat the mode is high and the curve short; i.e., many species occur in small numbers and none is very abundant. In a severe habitat the mode is low and the curve long; i. e., there are few rare species and a few with large numbers. This, again, seems to me to be an elaborate way of presenting data and to involve a lot of unnecessary arithmetic.

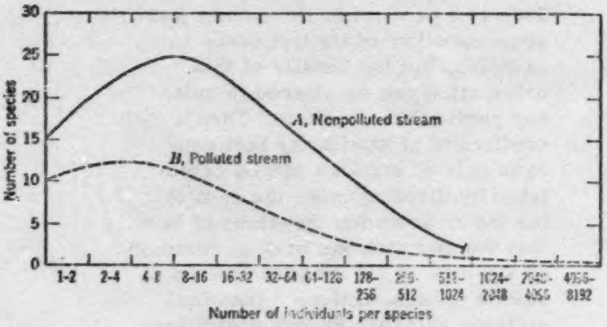

Figure 4. A graphic comparison of diatom communities from two different environments. (After Patrick et al., 1954) (30)

6 Diversity indices vs tabulated data

Allanson (1961) has applied this method to the invertebrate faunas of streams in South Africa and has shown, as has Patrick for diatoms, that the log normal curve is flatter and longer for polluted stations; the difference, however, is not so apparent that it does not need exposition. Here, again, I would suggest that tabulated data are just as informative. Indeed I would go further and say that tabulated data are essential in the present state of our knowledge. We are learning as we go along and if the details of the basic findings are concealed by some sort of arithmetical manipulation they cannot be re-interpreted in the light of later knowledge, nor are they preserved in the store of human knowledge. This point becomes particularly clear when one examines some of the early studies that include tables. Butcher (1946) requotes a considerable amount of data he collected from studies of various English rivers during the thirties; they are not only clear and easy to follow, but they are also informative about the generalities of pollution in a way that data quoted only within the confines of some particular system are not.

7 Examples of tabulated data(Table 5)

Simple tabulation of biological data in relation to water quality, either in terms of number of organisms, percentage composition of the biota, some arbitrary abundance scale, or as histograms, has been effectively practiced in many parts of the world: in America (Gaufin and Tarzwell 1952, Gaufin 1958), Africa (Harrison 1958 and 1960, Hynes and Williams 1962), Europe (Albrecht 1954, Kaiser 1951, Hynes 1961, Hynes and Roberts 1962), and New Zealand (Hirsch 1958) to cite a few. These tabulated data are easy to follow, are informative to the expert reader, and conceal no facts. Although the non-biologist may find them tedious, he need only read the explanatory paragraphs. It is a delusion to think that it is possible to reduce biological data to simple numerical levels. At best, these can only be produced for limited situations and

TABLE 5

ORGANISMS	1			2			3		
	206.3 power line			205.3			203.3		
	left	mid	right	left	mid	right	left	mid	right
Zoothamnium	F								
Dendrosoma	C								F
Spongilla fragilis								A	
Trochospongilla leidyi	C			C					
Unidentified Sponge					F			A	
Cordylophora lacustris		F		F	A	A			
Dugesia tigrina	3		2	4	4	6	14		19
Urnatella gracilis	C	C	C	C	F		F		F
Paludicella articulata	C			F	F	F	F		F
Fredericella sultana	C								
Pristina							1		
Nais communis				1		1		7	
Paranais									
Unidentified Leech									1
Unidentified Beetle			1						
Chaoborus punctipennis					1				
Hydrobaenus sp. A	31	47	5	9	137	68	1	26	4
Cricotopus bicinctus						1			
Unidentified Tendipedini								1	
Harnischia sp. A		5	2		29	2	1	8	
Tendipes nervosus	1				4	15		2	1
Tendipes modestus	59	14	16	7	12	7	1		3
Polypedilum sp. B									1
Calopsectra exigua			2	5	3	48	3	169	101
Tricorythodes	1			1			1		1
Stenonema				1					
Agraylea			2				1		
Athripsodes				2					
Potamyia flava				41		44	19	131	234
Hydropsyche orris				141		17	2	3	11
Cheumatopsyche		1		4	5	43		30	13
Psychomyiidae Genus A	51	22	21	30	54	92	11	193	21
Lithasia verrucosa									1
Ferrissia shimekii				4		33	7	5	19
Quadrula sp.						1	1		
Quadrula tuberculata						2			
Corbicula fluminea	4	1	5	11		1	77		7
TOTAL	152	90	56	261	229	381	141	575	437
	298			871			1,153		

F - few C - common A - abundant

Benthos from Pickwick Tailwater (35)

even then they need verbal exposition; at worst, they give a spurious impression of having absolute validity.

8 Comparison of stations

My final point in this section concerns comparisons. It is claimed that the German system, in effect, measures an absolute state, a definite level of water quality. We have seen that this is not a tenable claim. In the other systems, by and large, the need to establish local control stations at which to measure the normal or "natural" biotic conditions is accepted, and then other areas are compared with this supposed norm. This is, of course not always possible as there may remain no unaffected area, or no unaffected area that is, with respect to such factors as current, nature of substratum, etc., sufficiently similar to act as a base-line for data. Nevertheless, basically, these systems can be used to compare stations and thus to assess changes in water quality. In doing this, they can all be used more or less successfully, but I maintain that a table is just as useful as an elaborate analysis, and I believe that the table should be included with whatever is done. For a particular situation, however, it is often possible to distill the data into a single figure as a measure of similarity between stations.

9 Coefficients of similarity

Burlington (1962) and Dean and Burlington (1963) have recently proposed an entirely objective means of doing this, which involves simple arithmetical manipulation. In his system, a "prominence value" is calculated for each species at each station. This is a product of its density and some function of its frequency in samples, but the details of this calculation can be altered to suit any particular situation. Then a coefficient of similarity between each pair of stations can be calculated by dividing twice the sum of the lower prominence values of taxa that the two stations have in common by the sum of all the prominence values of both stations. Identical stations will then have a coefficient of similarity of 1:00; this coefficient will be lower the more the stations differ from one another. This is an easy way to compare stations in an entirely unbiased way and as such may satisfy the need for numerical exposition; however, it tells one nothing about why the localities are different and like all the other more or less numerical methods of presenting data has no absolute value. Moreover, it still leaves unanswered the fundamental question of how different is "different?"

TABLE 6

BIOTIC INDEX[1]

⇧ Clean

WATER QUALITY (high multiple use indicated)

Organisms in Order of Tendency to Disappear as Degree of Pollution Increases

⇩ Polluted

Types of Organisms Present	Variety Present	✓Total Number of Groups Present 0-1	2-5	6-10	11-15	16+
			Bio	Index		
Plecoptera nymph present	More than one species	—	7	8	9	10
	One species only	—	6	7	8	9
Ephemeroptera nymph present	More than one species *	—	6	7	8	9✱
	One species only*	—	5	6	7	8
Trichoptera larvae present	More than one species•	—	5	6	7	8
	One species only •	4	4	5	6	7
Gammaridae present	All above species absent	3	4	5	6	7
Asellus and/or Lirceus present	All above species absent	2	3	4	5	6
Tubificid worms, Tendipes, and Cricotopus bicinctus (one or more of these groups)	All above species absent	1	2	3	4	--
All above types absent	Some organisms such as Eristalis tenax not requiring dissolved oxygen may be present	0	1	2	--	--

*Stenonema nepotellum excluded

10✱ main stream reservoirs and west Tennessee streams

• Stenonema nepotellum (Ephem.) is counted in this section for the purpose of classification.

✓ ONE FOR EACH KNOWN SPECIES IN THESE GROUPS:

- Platyhelminthes
- Hirudinea
- Mollusca
- Crustacea
- Plecoptera
- Diptera (excluding specific ones listed below)
- Coleoptera
- Neuroptera

✓ ONE FOR EACH GROUP, REGARDLESS OF NUMBER OF SPECIES, ETC.:

- Annelida excluding Naididae
- Naididae
- Each Mayfly genera (excluding Stenonema nepotellum)
- Stenonema nepotellum
- Each Trichoptera family
- Chironomidae (excluding specific ones listed below)
- Chironomus riparius and plumosus and Cricotopus bicinctus.
- Family Simuliidae

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[1] adapted from Trent River Board - Tennessee Stream Pollution Control Board 8/66 RMS

IV THE PROBLEMS OF SAMPLING

The systems outlined above are all based on the assumption that it is possible to sample an aquatic habitat with some degree of accuracy; this is a dubious assumption, however, when applied to biological data. From what has been said about the complexity of biological reactions to the various factors in the environment, and from the obvious fact that rivers especially are a mosaic of microhabitats, it is clear that to achieve numerical accuracy or even some limits of confidence considerable numbers of samples need to be taken. Indeed, even in so apparently unvaried a habitat as a single riffle, Needham and Usinger (1956) showed that a very large number of samples would be necessary to give significant numerical data.

A Representative Sampling

There is a limit to the number of samples that can reasonably be taken and, anyway, it is desirable to sample many different types of habitat so as to get as broad as possible an estimate of the biota. This is the more recent approach of most of the workers in Central Europe, who have been content to cite abundances on a simple relative but arbitrary scale and to convert this to figures on some sort of logarithmic scale for use in calculations. An alternative is to express the catch in terms of percentage composition, but this had the disadvantage that micro- and macro-organisms cannot be expressed on the same scale as they are obtained by different collecting techniques. Also, of course, implicit in this approach is the assumption that the sampling is reasonably representative. Here again we run into the need for knowledge and expertise. In collection as well as in interpretation, the expert is essential. Biological sampling, unlike the simple, or fairly simple, filling of bottles for chemical analysis or the monitoring of measuring equipment, is a highly skilled job and not one to be handed over to a couple of vacationing undergraduates who are sent out with a Surber sampler and told to get on with it. This point has also been made by other biologists, e.g., Patrick (1961) who stresses the need for skilled and thorough collecting even for the determination of a species list.

B Non-Taxonomic Techniques

Alternatively we can use the less expert man when concentrating on only part of the habitat, using, say, microscopical slides suspended in the water to study algal growth. This method was extensively used by Butcher (1946), and Patrick et al. (1954) who studied diatoms in this way. This gives only a partial biological picture, but is useful as a means of monitoring a stretch of water where it is possible that changes might occur. It is a useful short-hand method, and as such is perhaps comparable to studying the oxygen absorbed from potassium permanganate instead of carrying out all the usual chemical analyses on water. A short method of this kind may serve very well most of the time, but, for instance, would not be likely to detect an insecticide in concentrations that could entirely eliminate arthropods and hence fishes by starvation.

C Monitoring

It is possible to work out biological monitoring systems for any specific purpose. The simplest of these is the cage of fish, which, like a single type of chemical analysis, can be expected to monitor only one thing — the ability of fish to live in the water — with no information on whether they can breed or whether there is anything for them to eat. Beak et al. (1959) describes a neat way in which the common constituents of the bottom fauna of Lake Ontario can be used to monitor the effluents from an industrial site. Obviously there is much room for such ingenuity in devising biological systems for particular conditions, but this is perhaps outside the scope of this meeting.

V CONCLUSIONS

It may appear from the previous sections that my attitude to this problem is entirely obstructionist. This is far from being so. Water quality is as much biological phenomenon as it is a chemical or physical one; often what we want to know about water is almost exclusively biological -- will it smell nasty, is it fit to drink, can one bathe in it, etc? I suggest, therefore, that it is desirable to organize water monitoring programs that will tell one what one wants to know. There is no point in measuring everything biological, just as there is no point in performing every possible chemical analysis; what is measured should be related to local conditions. It would be a waste of time to measure oxygen content in a clean mountain stream; we know it to be high, and it becomes worth measuring only if we suspect that it may have been lowered by pollution. Similarly, there is little point in studying the plankton in such a stream; we know it only reflects the benthic flora. In a lake or in a slow river, on the other hand, if our interest in the water lies in its potability, records of the plankton are of considerable importance as changes in plankton are, in fact, changes in the usability of the water.

A Periphyton and Benthos Studies

For long-term studies, especially for the recording of trends or changes induced by pollution, altered drainage, agricultural poisons, and other havoc wrought by man, one can expect informative results from two principal techniques: First, we can study microscopic plant and animal growth with glass slides placed in the water for fixed periods; second, we can obtain random samples of the benthic fauna. The algae and associated microfauna tell one a good deal about the nutrient condition of the water and the changes that occur in it, and the larger benthic fauna reveal changes in the trophic status, siltation due to soil erosion, effects of insecticides and other poisons, etc.

B Varying Levels of Complexity

The study of growths on glass slides is reasonably skilled work, but can easily be taught to technicians; like chemical monitoring, such study needs to be done fairly often. Sampling the benthos is more difficult and, as explained above, needs expert handling; unlike most other monitoring programs, however, it need be done only infrequently, say, once or twice a year. Inevitably sampling methods will vary with type of habitat; in each case, the question will arise as to whether it is worth looking at the fish also. It is here that the biologist must exercise judgment in devising and carrying out the sampling program.

C Data Interpretation

Judgment is also needed in the interpretation of the data. It is for this reason I maintain that it should all be tabulated so that it remains available for reassessment or comparison with later surveys. If need be, some sort of numerical format can be prepared from the data for ad hoc uses, but it should never become a substitute for tabulations. Only in this way can we go on building up our knowledge. Perhaps some day we shall be able to pass all this information into a computer, which will then be able to exercise better judgment than the biologist. I hope this will happen, as computers are better able to remember and to cope with complexity than men. It will not, however, pension off the biologist. He will still be needed to collect and identify the samples. I cannot imagine any computer wading about on rocky riffles nor persuading outboard motors and mechanical grabs to operate from the unstable confines of small boats. We shall still need flesh and blood biologists long after the advent of the hardware water chemist, even though, with reference to my earlier analogy, a Tokyo University computer recently outpointed 10 veteran medicals in diagnosing brain tumors and heart disease. It should be pointed out, however, that the computer still had to be fed with information, so we are still a long way from the hardware general practitioner. I believe though that he is likely to evolve before the hardware biologist; after all, he studies only one animal.

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Descriptors: Aquatic Life, Benthos, Water Quality, Environmental Effects, Biological Indices

APPLICATION OF BIOLOGICAL DATA

I ECOLOGICAL DATA HAS TRADITIONALLY BEEN DIVIDED INTO TWO GENERAL CLASSES:

A Qualitative - dealing with the taxonomic composition of communities

B Quantitative - dealing with the population density or rates of processes occurring in the communities

Each kind of data has been useful in its own way.

II QUALITATIVE DATA

A Certain species have been identified as:

1 Clean water (sensitive) or oligotrophic

2 Facultative, or tolerant

3 Preferring polluted regions (see: Fjerdinstad 1964, 1965; Gaufin & Tarzwell 1956; Palmer 1963, 1969; Rawson 1956; Teiling 1955)

B Using our knowledge about ecological requirements the biologist may compare the species present

1 At different stations in the same river (Gaufin 1958) or lake (Holland 1968)

2 In different rivers or lakes (Robertson and Powers 1967)

or changes in the species in a river or/lake over a period of several years. (Carr & Hiltunen 1965; Edmondson & Anderson 1956; Fruh, Stewart, Lee & Rohlich 1966; Hasler 1947).

C Until comparatively recent times taxonomic data were not subject to statistical treatment.

III QUANTITATIVE DATA: Typical Parameters of this type include:

A Counts - algae/ml; benthos/m^2; fish/net/day

B Volume - mm^3 algae/liter

C Weight - dry wgt; ash-free wgt.

D Chemical content - chlorophyll; carbohydrate; ATP; DNA; etc.

E Calories (or caloric equivalents)

F Processes - productivity; respiration

IV Historically, the chief use of statistics in treating biological data has been in the collection and analysis of samples for these parameters. Recently, many methods have been devised to convert taxonomic data into numerical form to permit:

A Better communication between the biologists and other scientific disciplines

B Statistical treatment of taxonomic data

C In the field of pollution biology these methods include:

1 Numerical ratings of organisms on the basis of their pollution tolerance

(saprobic valency: Zelinka & Sladecek 1964)

(pollution index: Palmer 1969)

2 Use of quotients or ratios of species in different taxonomic groups (Nygaard 1949)

3 Simple indices of community diversity:

a Organisms are placed in taxonomic groups which behave similarly under the same ecological conditions. The number of species in these groups found at "healthy" stations is compared to that found at "experimental" stations. (Patrick 1950)

b A truncated log normal curve is plotted on the basis of the number of individuals per diatom species. (Patrick, Hohn, & Wallace 1954)

c Sequential comparison index. (Cairns, Albough, Busey & Chanay 1968). In this technique, similar organisms encountered sequentially are grouped into "runs".

$$SCI = \frac{\text{runs}}{\text{total organisms examined}}$$

d Ratio of carotenoids to chlorophyll in phytoplankton populations:

OD_{430}/OD_{665} (Margalef 1968)

OD_{435}/OD_{670} (Tanaka, et al 1961)

e The number of diatom species present at a station is considered indicative of water quality or pollution level. (Williams 1964)

f $\frac{\text{number of species (S)}}{\text{number of individuals (N)}}$

g $\frac{\text{number of species (S)}}{\text{square root of number of individuals } (\sqrt{N})}$

h $\frac{S - 1}{\log_e N}$ (Menhinick 1964)

i $d = \frac{\Sigma n_i (n_i - 1)}{N (N - 1)}$ (Simpson 1949)

where n_i = number of individuals belonging to the i-th species, and

N = total number of individuals

j Information theory:

The basic equation used for information theory applications was developed by Margalef (1957).

$$I = \frac{1}{N} \log_2 \frac{N!}{N_a!\, N_b! \ldots N_s!}$$

where I - information/individual; $N_a, N_b \ldots N_s$ are the number of individuals in species a, b, ... s, and N is their sum.

This equation has also been used with:

1) The fatty acid content of algae (McIntire, Tinsley, and Lowry 1969)

2) Algal productivity (Dickman 1968)

3) Benthic biomass (Wilhm 1968)

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Descriptors: Analytical Techniques, Indicators

OPTICS AND THE MICROSCOPE

I OPTICS

An understanding of elementary optics is essential to the proper use of the microscope. The microscopist will find that unusual problems in illumination and photomicrography can be handled much more effectively once the underlying ideas in physical optics are understood.

A Reflection

A good place to begin is with reflection at a surface or interface. Specular (or regular) reflection results when a beam of light leaves a surface at the same angle at which it reached it. This type of reflection occurs with highly polished smooth surfaces. It is stated more precisely as Snell's Law, i.e., the angle of incidence, i, is equal to the angle of reflection, r (Figure 1). Diffuse (or scattered) reflection results when a beam of light strikes a rough or irregular surface and different portions of the incident light are reflected from the surface at different angles. The light reflected from a piece of white paper or a ground glass is an example of diffuse reflection.

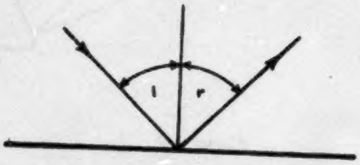


Figure 1

SPECULAR REFLECTION - SNELL'S LAW

Strictly speaking, of course, all reflected light, even diffuse, obeys Snell's Law. Diffuse reflected light is made up of many specularly reflected rays, each from a a tiny element of surface, and appears diffuse when the reflecting elements are very numerous and very small. The terms diffuse and specular, referring to reflection, describe not so much a difference in the nature of the reflection but rather a difference in the type of surface. A polished surface gives specular reflection; a rough surface gives diffuse reflection.

It is also important to note and remember that specularly reflected light tends to be strongly polarized in the plane of the reflecting surface. This is due to the fact that those rays whose vibration directions lie closest to the plane of the reflection surface are most strongly reflected. This effect is strongest when the angle of incidence is such that the tangent of the angle is equal to the refractive index of the reflecting surface. This particular angle of incidence is called the Brewster angle.

B Image Formation on Reflection

Considering reflection by mirrors, we find (Figure 2) that a plane mirror forms a virtual image behind the mirror, reversed right to left but of the same size as the object. The word virtual means that the image appears to be in a given plane but that a ground glass screen or a photographic film placed in that plane would show no image. The converse of a virtual image is a real image.

Spherical mirrors are either convex or concave with the surface of the mirror representing a portion of the surface of a sphere. The center of curvature is the center of the sphere, part of whose surface forms the mirror. The focus lies halfway between the center of curvature and the mirror surface.

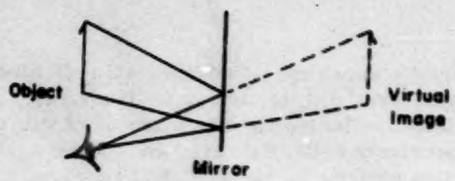


Figure 2

IMAGE FORMATION BY PLANE MIRROR

Construction of an image by a concave mirror follows from the two premises given below (Figure 3):

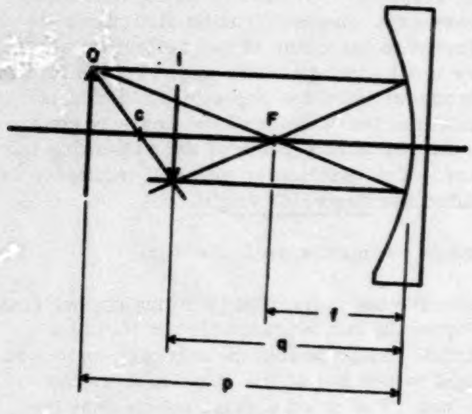


Figure 3

IMAGE FORMATION BY CONCAVE MIRROR

1 A ray of light parallel to the axis of the mirror must pass through the focus after reflection.

2 A ray of light which passes through the center of curvature must return along the same path.

A corollary of the first premise is:

3 A ray of light which passes through the focus is reflected parallel to the axis of the mirror.

The image from an object can be located using the familiar lens formula:

$$\frac{1}{p} + \frac{1}{q} = \frac{1}{f}$$

where p = distance from the object to the mirror
q = distance from the image to the mirror
f = focal length

C Spherical Aberration

No spherical surface can be perfect in its image-forming ability. The most serious of the imperfections, spherical aberration, occurs in spherical mirrors of large aperture (Figure 4). The rays of light making up an image point from the outer zone of a spherical mirror do not pass through the same point as the more central rays. This type of aberration is reduced by blocking the outer zone rays from the image area or by using aspheric surfaces.

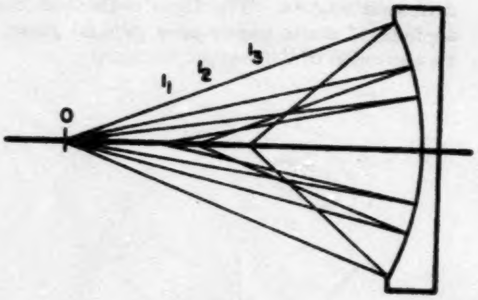


Figure 4

SPHERICAL ABERRATION BY SPHERICAL MIRROR

D Refraction of Light

Turning now to lenses rather than mirrors we find that the most important characteristic is refraction. Refraction refers to the change of direction and/or velocity of light as it passes from one medium to another. The ratio of the velocity in air (or more correctly in a vacuum) to the velocity in the medium is called the refractive index. Some typical values of refractive index measured with monochromatic light (sodium D line) are listed in Table 1.

Refraction causes an object immersed in a medium of higher refractive index than air to appear closer to the surface than it actually is (Figure 5). This effect may be used to determine the refractive index of a liquid with the microscope. A flat vial with a scratch on the bottom (inside) is placed on the stage of the microscope. The microscope is focused on the scratch and the fine adjustment micrometer reading is noted. A small amount of the unknown liquid is added; the scratch is again brought into focus and the new micrometer reading is taken. Finally, the microscope is refocused until the surface of the liquid appears in sharp focus. The micrometer reading is taken again and, with this information, the refractive index may be calculated from the simplified equation:

$$\text{refractive index} = \frac{\text{actual depth}}{\text{apparent depth}}$$

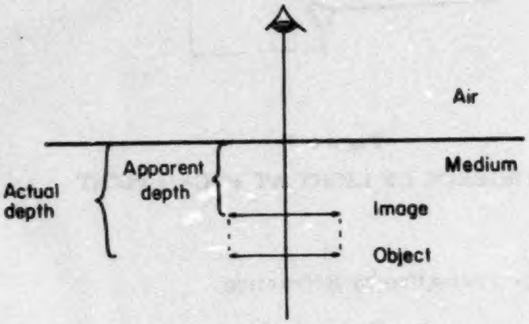


Figure 5

REFRACTION OF LIGHT AT INTERFACE

Table 1. REFRACTIVE INDICES OF COMMON MATERIALS MEASURED WITH SODIUM LIGHT

Vacuum	1.0000000	Crown glass	1.48 to 1.61
Air	1.0002918	Rock salt	1.5443
CO_2	1.0004498	Diamond	2.417
Water	1.3330	Lead sulfide	3.912

When the situation is reversed, and a ray of light from a medium of high refractive index passes through the interface of a medium of lower index, the ray is refracted until a critical angle is reached beyond which all of the light is reflected from the interface (Figure 6). This critical angle, C, has the following relationship to the refractive indices of the two media:

$$\sin C = \frac{n_2}{n_1}\text{, where } n_2 < n_1.$$

When the second medium is air, the formula becomes:

$$\sin C = \frac{1}{n_1}.$$

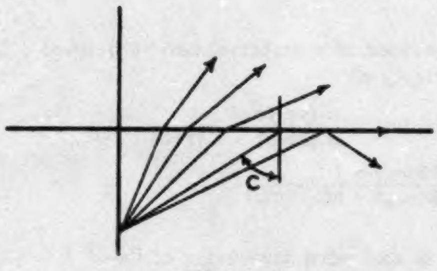


Figure 6

REFLECTION AT CRITICAL ANGLE

E Dispersion

Dispersion is another important property of transparent materials. This is the variation of refractive index with color (or wavelength) of light. When white light passes through a glass prism, the light rays are refracted by different amounts and separated into the colors of the spectrum. This spreading of light into its component colors is due to dispersion which, in turn, is due to the fact that the refractive index of transparent substances, liquids and solids, is lower for long wavelengths than for short wavelengths.

Because of dispersion, determination of the refractive index of a substance requires designation of the particular wavelength used. Light from a sodium lamp has a strong, closely spaced doublet with an average wavelength of 5893A, called the D line, which is commonly used as a reference wavelength. Table 2 illustrates the change of refractive index with wavelength for a few common substances.

Table 2. DISPERSION OF REFRACTIVE INDICES OF SEVERAL COMMON MATERIALS

	Refractive index		
	F line blue 4861A	D line (yellow) 5893A	C line (red) 6563A
Carbon disulfide	1.6523	1.6276	1.6182
Crown glass	1.5240	1.5172	1.5145
Flint glass	1.6391	1.6270	1.6221
Water	1.3372	1.3330	1.3312

The dispersion of a material can be defined quantitatively as:

$$\nu = \text{dispersion} = \frac{n\,(\text{yellow}) - 1}{n\,(\text{blue}) - n\,(\text{red})}$$

$$= \frac{n\,(593\text{m}\mu) - 1}{n\,(486\text{m}\mu) - n(656\text{m}\mu)}$$

where n is the refractive index of the material at the particular wavelength noted in the parentheses.

F Lenses

There are two classes of lenses, converging and diverging, called also convex and concave, respectively. The focal point of a converging lens is defined as the point at which a bundle of light rays parallel to the axis of the lens appears to converge after passing through the lens. The focal length of the lens is the distance from the lens to the focal point (Figure 7).

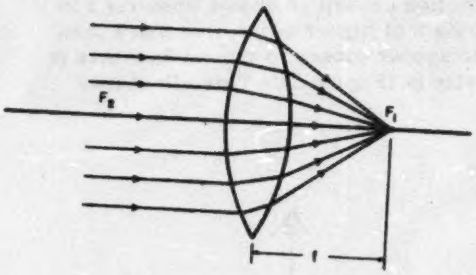


Figure 7

CONVERGENCE OF LIGHT AT FOCAL POINT

G Image Formation by Refraction

Image formation by lenses (Figure 8) follows rules analogous to those already given above for mirrors:

1 Light traveling parallel to the axis of the lens will be refracted so as to pass through the focus of the lens.

2 Light traveling through the geometrical center of the lens will be unrefracted.

The position of the image can be determined by remembering that a light ray passing through the focus, F, will be parallel to the axis of the lens on the opposite side of the lens and that a ray passing through the geometrical center of the lens will be unrefracted.

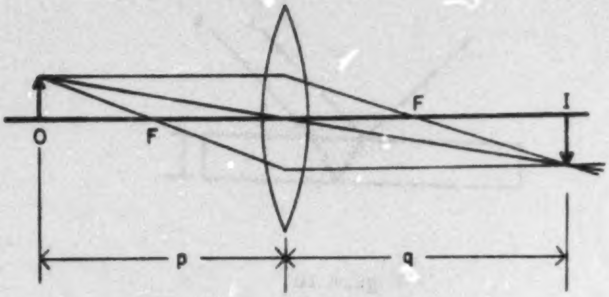


Figure 8

IMAGE FORMATION BY A CONVEX LENS

The magnification, M, of an image of an object produced by a lens is given by the relationship:

$$M = \frac{\text{image size}}{\text{object size}} = \frac{\text{image distance}}{\text{object distance}} = \frac{q}{p}$$

where q = distance from image to lens
and p = distance from object to lens.

H Aberrations of Lenses

Lenses have aberrations of several types which, unless corrected, cause loss of detail in the image. Spherical aberration appears in lenses with spherical surfaces. Reduction of spherical aberration can be accomplished by diaphragming the outer zones of the lens or by designing special aspherical surfaces in the lens system.

Chromatic aberration is a phenomenon caused by the variation of refractive index with wavelength (dispersion). Thus a lens receiving white light from an object will form a violet image closer to the lens and a red one farther away. Achromatic lenses are employed to minimize this effect. The lenses are combinations of two or more lens elements made up of materials having different dispersive powers. The use of monochromatic light is another obvious way of eliminating chromatic aberration.

Astigmatism is a third aberration of spherical lens systems It occurs when object points are not located on the optical axis of the lens and results in the formation of an indistinct image. The simplest remedy for astigmatism is to place the object close to the axis of the lens system.

I Interference Phenomena

Interference and diffraction are two phenomena which are due to the wave characteristics of light. The superposition of two light rays arriving simultaneously at a given point will give rise to interference effects, whereby the intensity at that point will vary from dark to bright depending on the phase differences between the two light rays.

The first requirement for interference is that the light must come from a single source. The light may be split into any number of paths but must originate from the same point (or coherent source). Two light waves from a coherent source arriving at a point in phase agreement will reinforce each other (Figure 9a). Two light waves from a coherent source arriving at a point in opposite phase will cancel each other (Figure 9b).

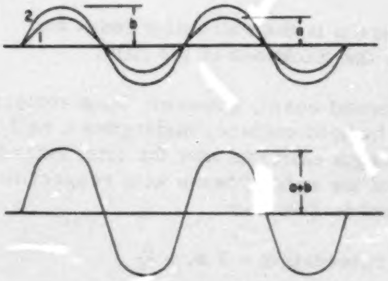


Figure 9a. Two light rays, 1 and 2, of the same frequency but different amplitudes, are in phase in the upper diagram. In the lower diagram, rays 1 and 2 interfere constructively to give a single wave of the same frequency and with an amplitude equal to the summation of the two former waves.

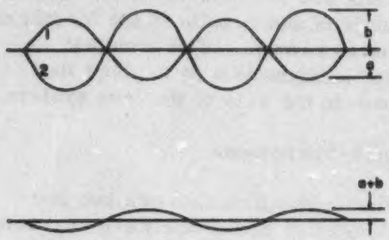


Figure 9b. Rays 1 and 2 are now 180° out of phase and interfere destructively. The resultant, in the bottom diagram, is of the same frequency but is of reduced amplitude (a is negative and is subtracted from b).

The reflection of a monochromatic light beam by a thin film results in two beams, one reflected from the top surface and one from the bottom surface. The distance traveled by the latter beam in excess of the first is twice the thickness of the film and its equivalent air path is:

$$2\,nt$$

where n is the refractive index and t is the thickness of the film.

The second beam, however, upon reflection at the bottom surface, undergoes a half wavelength shift and now the total retardation of the second beam with respect to the first is given as:

$$\text{retardation} = 2\,nt + \frac{\lambda}{2}$$

where λ is the wavelength of the light beam.

When retardation is exactly an odd number of half wavelengths, destructive interference takes place resulting in darkness. When it is zero or an even number of half wavelengths, constructive interference results in brightness (Figure 10).

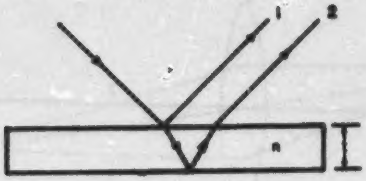


Figure 10

INTERFERENCE IN A THIN FILM

A simple interferometer can be made by partially silvering a microscope slide and cover slip. A preparation between the two partially silvered surfaces will show interference fringes when viewed with monochromatic light, either transmitted or by vertical illuminator. The fringes will be close together with a wedge-shaped preparation and will reflect refractive index differences due to temperature variations, concentration differences, different solid phases, etc. The method has been used to measure quantitatively the concentration of solute around a growing crystal(1) (Figure 11).

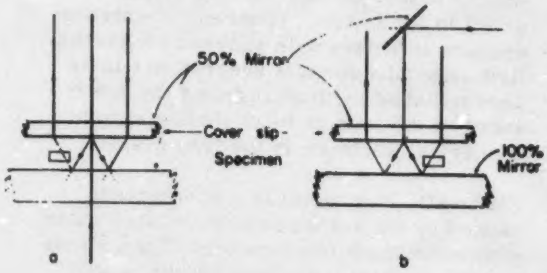


Figure 11

MICROSCOPICAL METHOD OF VIEWING INTERFERENCE IMAGES

a Examination is by transmitted light. Light ray undergoes multiple reflections and produces dark and light fringes in the field. A specimen introduces a phase shift and changes the fringe pattern.

b Illumination is from the top. The principle is the same but fringes show greater contrast.

Each dark band represents an equivalent air thickness of an odd number of half wavelengths. Conversely, each bright band is the result of an even number of half wavelengths.

With interference illumination, the effect of a transparent object of different refractive index than the medium in the microscope field is:

1 a change of light intensity of the object if the background is uniformly illuminated (parallel cover slip), or

2 a shift of the interference bands within the object if the background consists of bands (tilted cover slip).

The relationship of refractive indices of the surrounding medium and the object is as follows:

$$n_s = n_m\left(1 + \frac{\theta\lambda}{360t}\right)$$

where n_s = refractive index of the specimen
n_m = refractive index of the surrounding medium
θ = phase shift of the two beams, degrees
λ = wavelength of the light
t = thickness of the specimen.

J Diffraction

In geometrical optics, it is assumed that light travels in straight lines. This is not always true. We note that a beam passing through a slit toward a screen creates a bright band wider than the slit with alternate bright and dark bands appearing on either side of the central bright band, decreasing in intensity as a function of the distance from the center. Diffraction describes this phenomenon and, as one of its practical consequences, limits the lens in its ability to reproduce an image. For example, the image of a pin point of light produced by a lens is not a pin point but is revealed to be a somewhat larger patch of light surrounded by dark and bright rings. The diameter, d, of this diffraction disc (to the first dark ring) is given as:

$$d = \frac{2.44\, f\lambda}{D}$$

where f is the focal length of the lens, λ the wavelength, and D the diameter of the lens.

It is seen that in order to maintain a small diffraction disc at a given wavelength, the diameter of the lens should be as large as possible with respect to the focal length. It should be noted, also, that a shorter wavelength produces a smaller disc.

If two pin points of light are to be distinguished in an image, their diffraction discs must not overlap more than one half their diameters. The ability to distinguish such image points is called resolving power and is expressed as one half of the preceding expression:

$$\text{resolving power} = \frac{1.22\, f\lambda}{D}.$$

II THE COMPOUND MICROSCOPE

The compound microscope is an extension in principle of the simple magnifying glass; hence it is essential to understand fully the properties of this simple lens system.

A Image Formation by the Simple Magnifier

The apparent size of an object is determined by the angle that is formed at the eye by the extreme rays of the object. By bringing the object closer to the eye, that angle (called the visual angle) is increased. This also increases the apparent size. However a limit of accommodation of the eye is reached, at which distance the eye can no longer focus. This limiting distance is about 10 inches or 25 centimeters. It is at this distance that the magnification of an object observed by the unaided eye is said to be unity. The eye can, of course, be focused at shorter distances but not usually in a relaxed condition.

A positive, or converging, lens can be used to permit placing an object closer than 10 inches to the eye (Figure 12). By this means the visual angle of the object is increased (as is its apparent size) while the image of

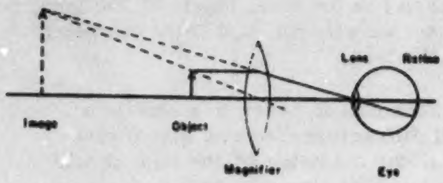


Figure 12

VIRTUAL IMAGE FORMATION BY CONVEX LENS

the object appears to be 10 inches from the eye, where it is best accommodated.

B Magnification by a Single Lens System

The magnification, M, of a simple magnifying glass is given by:

$$M = \frac{25}{f} + 1$$

where f = focal length of the lens in centimeters.

Theoretically the magnification can be increased with shorter focal length lenses. However such lenses require placing the eye very close to the lens surface and have much image distortion and other optical aberrations. The practical limit for a simple magnifying glass is about 20X.

In order to go to magnifications higher than 20X, the compound microscope is required. Two lens systems are used to form an enlarged image of an object (Figure 13). This is accomplished in two steps, the first by a lens called the objective and the second by a lens known as the eyepiece (or ocular).

C The Objective

The objective is the lens (or lens system) closest to the object. Its function is to reproduce an enlarged image of the object in the body tube of the microscope. Objectives are available in various focal lengths to give different magnifications (Table 3). The magnification is calculated from the focal length by dividing the latter into the tube length, usually 160 mm.

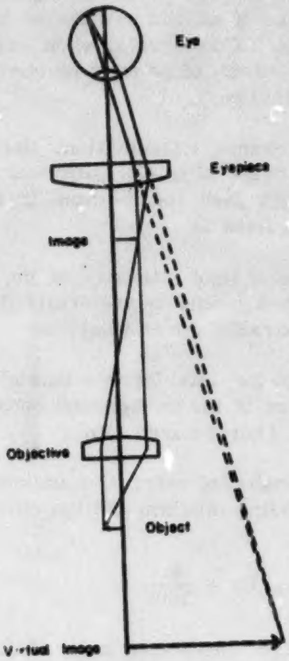


Figure 13

IMAGE FORMATION IN COMPOUND MICROSCOPE

The numerical aperture (N. A.) is a measure of the ability of an objective to resolve detail. This is more fully discussed in the next section. The working distance is in the free space between the objective and the cover slip and varies slightly for objectives of the same focal length depending upon the degree of correction and the manufacturer.

There are three basic classifications of objectives: achromats, fluorites and apochromats, listed in the order of their complexity. The achromats are good for routine work while the fluorites and apochromats offer additional optical corrections to compensate for spherical, chromatic and other aberrations.

Table 3. NOMINAL CHARACTERISTICS OF USUAL MICROSCOPE OBJECTIVES

Nominal focal length mm	Nominal magnif.	N. A.	Working distance mm	Depth focus µ	Diam. of field mm.	Resolving power, white light, µ	Maximum useful magnif.	Eyepiece for max. useful magnif.
56	2.5X	0.08	40	50	8.5	4.4	80X	30X
32	5	0.10	25	16	5	3.9	90X	20X
16	10	0.25	7	8	2	1.4	250X	25X
8	20	0.50	1.3	2	1	0.7	500X	25X
4	43	0.66	0.7	1	0.5	0.4	660X	15X
4	45	0.85	0.5	1	0.4	0.35	850X	20X
1.8	90	1.30	0.2	0.4	0.2	0.21	1250X	12X

Another system of objectives employs reflecting surfaces in the shape of concave and convex mirrors. Reflection optics, because they have no refracting elements, do not suffer from chromatic aberrations as ordinary refraction objectives do. Based entirely on reflection, reflecting objectives are extremely useful in the infrared and ultraviolet regions of the spectrum. They also have a much longer working distance than the refracting objectives.

The body tube of the microscope supports the objective at the bottom (over the object) and the eyepiece at the top. The tube length is maintained at 160 mm except for Leitz instruments, which have a 170-mm tube length.

The objective support may be of two kinds, an objective clutch changer or a rotating nosepiece:

1 The objective clutch changer ("quick-change" holder) permits the mounting of only one objective at a time on the microscope. It has a centering arrangement, so that each objective need be centered only once with respect to the stage rotation. The changing of objectives with this system is somewhat awkward compared with the rotating nosepiece.

2 The revolving nosepiece allows mounting three or four objectives on the microscope at one time (there are some nosepieces that accept five and even six objectives). In this system, the objectives are usually noncenterable and the stage is centerable. Several manufacturers provide centerable objective mounts so that each objective on the nosepiece need be centered only once to the fixed rotating stage. The insides of objectives are better protected from dust by the rotating nosepiece. This, as well as the inconvenience of the so-called "quick-change" objective holder, makes it worthwhile to have one's microscope fitted with rotating nosepiece.

D The Ocular

The eyepiece, or ocular, is necessary in the second step of the magnification process. The eyepiece functions as a simple magnifier viewing the image formed by the objective.

There are three classes of eyepieces in common use: huyghenian, compensating and flat-field. The huyghenian (or huyghens) eyepiece is designed to be used with achromats while the compensating type is used with fluorite and apochromatic objectives. Flat-field eyepieces, as the name implies, are employed in photomicrography or projection and can be used with most objectives. It is best to follow the recommendations of the manufacturer as to the proper combination of objective and eyepiece.

The usual magnifications available in oculars run from about 6X up to 25 or 30X. The 6X is generally too low to be of any real value while the 25 and 30X oculars have slightly poorer imagery than medium powers and have a very low eyepoint. The most useful eyepieces lie in the 10 to 20X magnification range.

E Magnification of the Microscope

The total magnification of the objective-eyepiece combination is simply the product of the two individual magnifications. A convenient working rule to assist in the proper choice of eyepieces states that the maximum useful magnification (MUM) for the microscope is 1,000 times the numerical aperture (N.A.) of the objective. The MUM is related to resolving power in that magnification in excess of MUM gives little or no additional resolving power and results in what is termed *empty magnification*. Table 4 shows the results of such combinations and a comparison with the 1000X N.A. rule. The underlined figure shows the magnification nearest to the MUM and the eyepiece required with each objective to achieve the MUM. From this table it is apparent that only higher power eyepieces can give full use of the resolving power of the objectives. It is obvious that a 10X, or even a 15X, eyepiece gives insufficient magnification for the eye to see detail actually resolved by the objective.

F Focusing the Microscope

The coarse adjustment is used to roughly position the body tube (in some newer microscopes, the stage) to bring the image into focus. The fine adjustment is used after the coarse adjustment to bring the image into perfect focus and to maintain the focus as the slide is moved across the stage. Most microscope objectives are parfocal so that once they are focused any other objective can be swung into position without the necessity of refocusing except with the fine adjustment.

The student of the microscope should first learn to focus in the following fashion, to prevent damage to a specimen or objective:

1 Raise the body tube and place the specimen on the stage.

2 Never focus the body tube down (or the stage up) while observing the field through the eyepiece.

3 Lower the body tube (or raise the stage) with the coarse adjustment while carefully observing the space between the

Table 4. MICROSCOPE MAGNIFICATION CALCULATED FOR VARIOUS OBJECTIVE-EYEPIECE COMBINATIONS

Objective		Eyepiece					MUM[a] (1000 NA)
Focal length	Magnification	5X	10X	15X	20X	25X	
56mm	3X	15X	30X	45X	60X	75X	80X
32	5	25X	50X	75X	100X	125X	100X
16	10	50X	100X	150X	200X	250X	250X
8	20	100X	200X	300X	400X	500X	500X
4	40	200X	400X	600X	800X	1000X	660X
1.8	90	450X	900X	1350X	1800X	2250X	1250X

[a] MUM = maximum useful magnification

objective and slide and permitting the two to come close together without touching.

4 Looking through the microscope and turning the fine adjustment in such a way as to move the objective away from the specimen, bring the image into sharp focus.

The fine adjustment is usually calibrated in one- or two-micron steps to indicate the vertical movement of the body tube. This feature is useful in making depth measurements but should not be relied upon for accuracy.

G The Substage Condenser

The substage holds the condenser and polarizer. It can usually be focused in a vertical direction so that the condenser can be brought into the correct position with respect to the specimen for proper illumination. In some models, the condenser is centerable so that it may be set exactly in the axis of rotation of the stage; otherwise it will have been precentered at the factory and should be permanent.

H The Microscope Stage

The stage of the microscope supports the specimen between the condenser and objective, and may offer a mechanical stage as an attachment to provide a means of moving the slide methodically during observation. The polarizing microscope is fitted with a circular rotating stage to which a mechanical stage may be added. The rotating stage, which is used for object orientation to observe optical effects, will have centering screws if the objectives are not centerable, or vice versa. It is undesirable to have both objectives and stage centerable as this does not provide a fixed reference axis.

I The Polarizing Elements

A polarizer is fitted to the condenser of all polarizing microscopes. In routine instruments, the polarizer is fixed with its vibration direction oriented north-south (east-west for most European instruments) while in research microscopes, the polarizer can be rotated. Modern instruments have polarizing filters (such as Polaroid) replacing the older calcite prisms. Polarizing filters are preferred because they:

1 are low-cost;

2 require no maintenance;

3 permit use of the full condenser aperture.

An analyzer, of the same construction as the polarizer, is fitted in the body tube of the microscope on a slider so that it may be easily removed from the optical path. It is oriented with its plane of vibration perpendicular to the corresponding direction of the polarizer.

J The Bertrand Lens

The Bertrand lens is usually found only on the polarizing microscope although some manufacturers are beginning to include it on phase microscopes. It is located in the body tube above the analyzer on a slider (or pivot) to permit quick removal from the optical path. The Bertrand lens is used to observe the back focal plane of the objective. It is convenient for checking quickly the type and quality of illumination, for observing interference figures of crystals, for adjusting the phase annuli in phase microscopy and for adjusting the annular and central stops in dispersion staining.

K The Compensator Slot

The compensator slot receives compensators (quarter-wave, first-order red and quartz-wedge) for observation of the optical properties of crystalline materials. It is usually placed at the lower end of the body tube just above the objective mount, and is oriented 45° from the vibration directions of the polarizer and analyzer.

L The Stereoscopic Microscope

The stereoscopic microscope, also called the binocular, wide-field, dissecting or

Greenough binocular microscope, is in reality a combination of two separate compound microscopes. The two microscopes, usually mounted in one body, have their optical axes inclined from the vertical by about 7^{o} and from each other by twice this angle. When an object is placed on the stage of a stereoscopic microscope, the optical systems view it from slightly different angles, presenting a stereoscopic pair of images to the eyes, which fuse the two into a single three-dimensional image.

The objectives are supplied in pairs, either as separate units to be mounted on the microscope or, as in the new instruments, built into a rotating drum. Bausch and Lomb was the first manufacturer to have a zoom lens system which gives a continuous change in magnification over the full range. Objectives for the stereomicroscope run from about 0.4X to 12X, well below the magnification range of objectives available for single-objective microscopes.

The eyepieces supplied with stereoscopic microscopes run from 10 to 25X and have wider fields than their counterparts in the single-objective microscopes.

Because of mechanical limitations, the stereomicroscope is limited to about 200X magnification and usually does not permit more than about 120X. It is most useful at relatively low powers in observing shape and surface texture, relegating the study of greater detail to the monocular microscope. The stereomicroscope is also helpful in manipulating small samples, separating ingredients of mixtures, preparing specimens for detailed study at higher magnifications and performing various mechanical operations under microscopical observation, e. g. micromanipulation.

III ILLUMINATION AND RESOLVING POWER

Good resolving power and optimum specimen contrast are prerequisites for good microscopy. Assuming the availability of suitable optics (ocular, objectives and substage condenser) it is still of paramount importance to use proper illumination. The requirement for a good illumination system for the microscope is to have uniform intensity of illumination over the entire field of view with independent control of intensity and of the angular aperture of the illuminating cone.

A Basic Types of Illumination

There are three types of illumination (Table 5) used generally:

1 Critical. This is used when high levels of illumination intensity are necessary for oil immersion, darkfield, fluorescence, low birefringence or photomicrographic studies. Since the lamp filament is imaged in the plane of the specimen, a ribbon filament or arc lamp is required. The lamp must be focusable and have an iris diaphragm; the position of the filament must also be adjustable in all directions.

2 Köhler. Also useful for intense illumination, Köhler illumination may be obtained with any lamp not fitted with a ground glass. The illuminator must, however, be focusable, it must have an adjustable field diaphragm (iris) and the lamp filament position must be adjustable in all directions.

3 "Poor man's". So-called because a low-priced illuminator may be used, this method gives illumination of high quality although of lower intensity because of the presence of a ground glass in the system. No adjustments are necessary on the illuminator or lamp filament although an adjustable diaphragm on the illuminator is helpful.

All three types of illumination require that the microscope substage condenser focus the image of the illuminator aperture in the plane of the specimen. In each case, then, the lamp iris acts as a field diaphragm and should be closed to just illuminate the field of view. The differences in these three types of illumination lie in the adjustment of the lamp condensing lens. With poor man's illumination there is no lamp condenser, hence no adjustment. The lamp should be placed close to the microscope so that

Table 5. COMPARISON OF CRITICAL, KOHLER AND POOR MAN'S ILLUMINATION

	Critical	Köhler	Poor man's
Lamp filament	ribbon filament	any type	any type
Lamp condensing lens	required	required	none
Lamp iris	required	required	useful
Ground glass at lamp	none	none	present
Image of light source	in object plane	at substage iris	none
Image of field iris	near object plane	in object plane	near object plane
Image of substage iris	back focal plane of objective	back focal plane of objective	back focal plane of objective

the entire field of view is always illuminated. If the surface structure of the ground glass becomes apparent in the field of view the substage condenser is very slightly defocused.

Critical Illumination

With critical illumination the lamp condenser is focused to give parallel rays; focusing the lamp filament on a far wall is sufficient. Aimed, then, at the substage mirror, the substage condenser will focus the lamp filament in the object plane. The substage condenser iris will now be found imaged in the back focal plane of the objective; it serves as a control over convergence of the illumination. Although the substage iris also affects the light intensity over the field of view it should most decidedly not be used for this purpose. The intensity of illumination may be varied by the use of neutral density filters and, unless color photomicrography is anticipated, by the use of variable voltage on the lamp filament.

Köhler illumination (Figure 14) differs from critical illumination in the use of the lamp condenser. With critical illumination the lamp condenser focuses the lamp filament at infinity; with Kohler illumination the lamp filament is focused in the plane of the substage condenser iris (also coincident with the anterior focal plane of the substage condenser). The functions of the lamp condenser iris and the substage condenser iris in controlling, respectively, the area of the illuminated field of view and the angular aperture of the illuminating cone are precisely alike for all three types of illumination.

Critical illumination is seldom used because it requires a special lamp filament and because, when used, it shows no advantage over well-adjusted Köhler illumination.

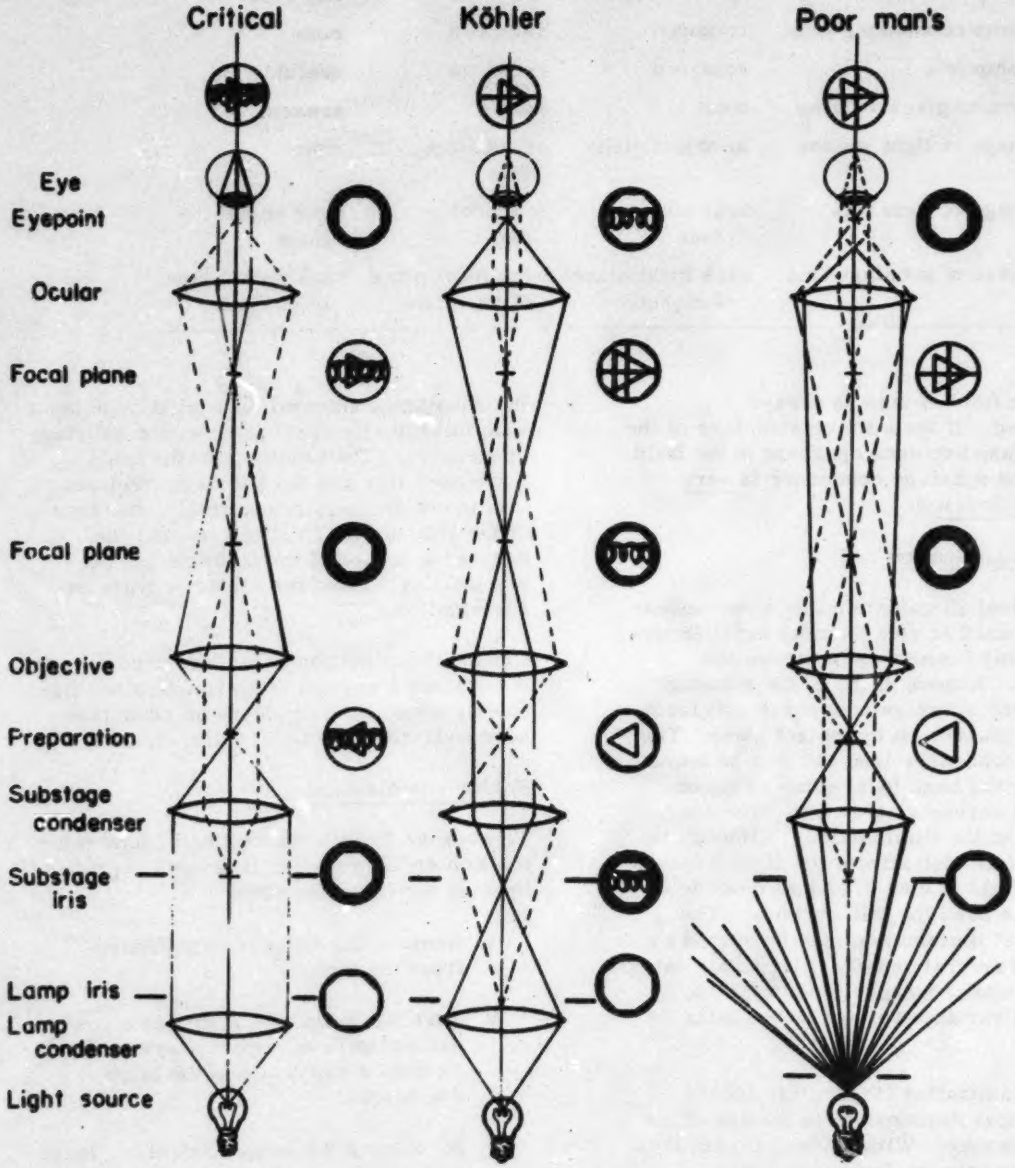
Köhler Illumination

To arrange the microscope and illuminator for Köhler illumination it is well to proceed through the following steps:

a Remove the diffusers and filters from the lamp.

b Turn the lamp on and aim at a convenient wall or vertical screen about 19 inches away. Open the lamp diaphragm.

c By moving the lamp condenser, focus a sharp image of the filament. It should be of such a size as to fill, not necessarily evenly, the microscope


Critical
Köhler
Poor man's
Eye
Eyepoint
Ocular
Focal plane
Focal plane
Objective
Preparation
Substage condenser
Substage iris
Lamp iris
Lamp condenser
Light source

substage condenser opening. If it does not, move the lamp away from the wall to enlarge the filament image; refocus.

d Turn the lamp and aim it at the microscope mirror so as to maintain the same 18 inches (or adjusted lamp distance).

e Place a specimen on the microscope stage and focus sharply with a 16-mm (10X) objective. Open fully the aperture diaphragm in the substage condenser. If the light is too bright, temporarily place a neutral density filter or a diffuser in the lamp.

f Close the lamp diaphragm, or field diaphragm, to about a 1-cm opening. Rack the microscope substage condenser up and down to focus the field diaphragm sharply in the same plane as the specimen.

g Adjust the mirror to center the field diaphragm in the field of view.

h Remove the 16-mm objective and replace with a 4-mm objective. Move the specimen so that a clear area is under observation. Place the Bertrand lens in the optical path, or remove the eyepiece and insert an auxiliary telescope (sold with phase contrast accessories) in its place, or remove the eyepiece and observe the back aperture of the objective directly. Remove any ground glass diffusers from the lamp. Now observe the lamp filament through the microscope.

i If the filament does not appear to be centered, swing the lamp housing in a horizontal arc whose center is at the field diaphragm. The purpose is to maintain the field diaphragm on the lamp in its centered position. If a vertical movement of the filament is required, loosen the bulb base and slide it up or down. If the base is fixed, tilt the lamp housing in a vertical arc with the field diaphragm as the center of movement (again endeavoring to keep the lamp diaphragm in the centered position). If you have mastered this step, you have accomplished the most difficult portion. (Better microscope lamps have adjustments to move the bulb independently of the lamp housing to simplify this step.)

j Put the specimen in place, replace the eyepiece and the desired objective and refocus.

k Open or close the field diaphragm until it just disappears from the field.

l Observe the back aperture of the objective, preferably with the Bertrand lens or the auxiliary telescope, and close the aperture diaphragm on the substage condenser until it is about four-fifths the diameter of the back aperture. This is the best position for the aperture diaphragm, a position which minimizes glare and maximizes the resolving power. It is instructive to vary the aperture diaphragm and observe the image critically during the manipulation.

m If the illumination is too great, insert an appropriate neutral density filter between the illuminator and the condenser. Do not use the condenser aperture diaphragm or the lamp field diaphragm to control the intensity of illumination.

Poor Man's Illumination

Both critical and Köhler illumination require expensive illuminators with adjustable focus, lamp iris and adjustable lamp mounts. Poor man's illumination requires a cheap illuminator although an expensive illuminator may be used if its expensive features are negated by inserting a ground glass diffuser or by using a frosted bulb. Admittedly an iris diaphragm on the lamp would be a help though it is not necessary.

a The illuminator must have a frosted bulb or a ground glass diffuser.

It should be possible to direct it in the general direction of the substage mirror, very close thereto or in place thereof.

b Focus on any preparation after tilting the mirror to illuminate the field.

c Remove the top lens of the condenser and, by racking the condenser up or, more often, down, bring into focus (in the same plane as the specimen) a finger, pencil or other object placed in the same general region as the ground glass diffuser on the lamp. The glass surface itself can then be focused in the plane of the specimen.

d Ideally the ground glass surface will just fill the field of view when centered by the substage mirror; adjustment may be made by moving the lamp closer to or farther from the microscope (the position might be marked for each objective used) or by cutting paper diaphragms of fixed aperture (one for each objective used). In this instance a lamp iris would be useful.

e Lower the condenser just sufficiently to defocus the ground glass surface and render the field of illumination even.

f Observe the back aperture of the objective and open the substage condenser iris about 75 percent of the way. The final adjustment of the substage iris is made while observing the preparation; the iris should be open as far as possible, still giving good contrast.

g The intensity of illumination should be adjusted only with neutral density filters or by changing the lamp voltage.

Proper illumination is one of the most important operations in microscopy. It is easy to judge a microscopist's ability by a glance at his field of view and the objective back lens.

B Resolving Power

The resolving power of the microscope is its ability to distinguish separate details of closely spaced microscopic structures. The theoretical limit of resolving two discrete points, a distance X apart, is:

$$X = \frac{1.22\lambda}{2\ N.A.}$$

where λ = wavelength of light used to illuminate the specimen

N. A. = numerical aperture of the objective

Substituting a wavelength of 4,500 Angstroms and a numerical aperture of 1.3, about the best that can be done with visible light, we find that two points about 2,000A (or 0.2 micron) apart can be seen as two separate points. Further increase in resolving power can be achieved for the light microscope by using light or shorter wavelength. Ultraviolet light near 2,000 Angstroms lowers the limit to about 0.1 micron, the lower limit for the light microscope.

The numerical aperture of an objective is usually engraved on the objective and is related to the angular aperture, AA (Figure 15), by the formula:

$$N.A. = n \sin \frac{AA}{2}$$

where n = the lowest index in the space between the object and the objective.

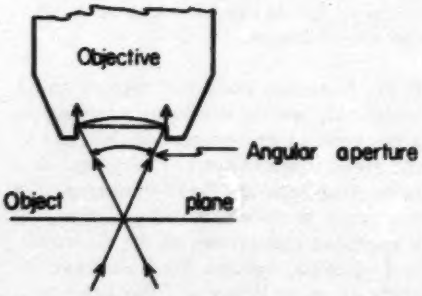


Figure 15

ANGULAR APERTURE OF MICROSCOPE OBJECTIVE

1 Maximum useful magnification

A helpful rule of thumb is that the useful magnification will not exceed 1,000 times the numerical aperture of the objective (see Tables 3 and 4). Although somewhat higher magnification may be used in specific cases, no additional detail will be resolved.

It is curious, considering the figures in the table, that most, if not all, manufacturers of microscopes furnish a 10X eyepiece as the highest power. A 10X eyepiece is useful but anyone interested in critical work should use a 15-25X eyepiece; the 5-10X eyepieces are best for scanning purposes.

2 Abbe's theory of resolution

One of the most cogent theories of resolution is due to Ernst Abbe, who suggested that microscopic objects act like diffraction gratings (Figure 16) and that the angle of diffraction, therefore, increases with the fineness of the detail. He proposed that a given microscope objective would resolve a particular detail if at least two or three transmitted rays (one direct and two diffracted rays) entered the objective. In Figure 16 the detail shown would be resolved in A and C but not in B. This theory, which can be borne out by simple experiment, is useful in showing how to improve resolution. Since shorter wavelengths will give a smaller diffraction angle, there is more chance of resolving fine detail with short wavelengths. Also, since only two of the transmitted rays are needed, oblique light and a high N.A. condenser will aid in resolving fine detail.

3 Improving resolving power

The following list summarizes the practical approaches to higher resolution with the light microscope:

a The specimen should be illuminated by either critical or Köhler illumination.

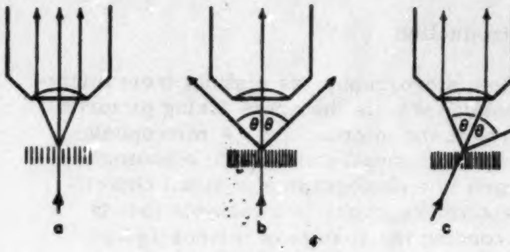


Figure 16

ABBE THEORY OF RESOLUTION

b The condenser should be well-corrected and have a numerical aperture as high as the objective to be used.

c An apochromatic oil-immersion objective should be used with a compensating eyepiece of at least 15X magnification. The immersion oil should have an index close to 1.515 and have proper dispersion for the objective being used.

d Immersion oil should be placed between the condenser and slide and between cover slip and objective. The preparation itself should be surrounded by a liquid having a refractive index of 1.515 or more.

e The illumination should be reasonably monochromatic and as short in wavelength as possible. An interference filter transmitting a wavelength of about 480-500 millimicrons is a suitable answer to this problem. Ideally, of course, ultraviolet light should be used to decrease the wavelength still further.

The practical effect of many of these factors is critically discussed by Loveland(2) in a paper on the optics of object space.

IV PHOTOMICROGRAPHY

A Introduction

Photomicrography, as distinct from microphotography, is the art of taking pictures through the microscope. A microphotograph is a small photograph; a photomicrograph is a photograph of a small object. Photomicrography is a valuable tool in recording the results of microscopical study. It enables the microscopist to:

1 describe a microscopic field objectively without resorting to written descriptions,

2 record a particular field for future reference,

3 make particle size counts and counting analyses easily and without tying up a microscope,

4 enhance or exaggerate the visual microscopic field to bring out or emphasize certain details not readily apparent visually,

5 record images in ultraviolet and infrared microscopy which are otherwise invisible to the unaided eye.

There are two general approaches to photomicrography; one requires only a plate or film holder supported above the eyepiece of the microscope with a light-tight bellows; the other utilizes any ordinary camera with its own lens system, supported with a light-tight adaptor above the eyepiece. It is best, in the latter case, to use a reflex camera so that the image can be carefully focused on the ground glass. Photomicrography of this type can be regarded simply as replacing the eye with the camera lens system. The camera should be focused at infinity, just as the eye is for visual observation, and it should be positioned close to and over the eyepiece.

The requirements for photomicrography, however, are more rigorous than those for visual work. The eye can normally compensate for varying light intensities, curvature of field and depth of field. The photographic plate, however, lies in one plane; hence the greatest care must be used to focus sharply on the subject plane of interest and to select optics to give minimum amounts of field curvature and chromatic aberrations.

With black and white film, color filters may be used to enhance the contrast of some portions of the specimen while minimizing chromatic aberrations of the lenses. In color work, however, filters cannot usually be used for this purpose and better optics may be required.

Photomicrographic cameras which fit directly onto the microscope are available in 35-mm or up to 3-1/4 × 4-1/4 inch sizes. Others are made which accommodate larger film sizes and which have their own support independent of the microscope. The former, however, are preferred for ease of handling and lower cost. The latter system is preferred for greater flexibility and versatility and lack of vibration. The Polaroid camera has many applications in microscopy and can be used on the microscope directly but, because of its weight, only when the microscope has a vertically moving stage for focusing rather than a focusing body tube.

B Determination of Correct Exposure

Correct exposure determination can be accomplished by trial and error, by relating new conditions to previously used successful conditions and by photometry.

With the trial and error method a series of trial exposures is made, noting the type of subject, illumination, filters, objective, eyepiece, magnification, film and shutter speed. The best exposure is selected. The following parameters can be changed and the exposure time adjusted accordingly:

1 Magnification. Exposure time varies as the square of the magnification.

Example: Good exposure was obtained with a 1/10-second exposure and a magnification of 100X. If the magnification is now

200X, the correct exposure is calculated as follows:

$$\text{new exposure time} = \text{old exposure time}$$

$$\times \left(\frac{\text{new magnification}}{\text{old magnification}}\right)^2 = 1/10 \left(\frac{200}{100}\right)^2 =$$

4/10 or, say, 1/2 second.

It should be noted, however, that the above calculation can be made only when there has been no change in the illumination system including the condenser or the objective. Only changes in magnification due to changing eyepieces or bellows extension distance can be handled in the above manner.

2 Numerical aperture. Exposure time varies inversely as the square of the smallest working numerical aperture of the condenser and objective.

Example: Good exposure was obtained at 1/10 second with the 10X objective, N. A. 0.25, at full aperture. With a 20X objective, N. A. 0.25, at full aperture and the same final magnification, what is the correct exposure time?

$$\text{new exposure time} = \text{old exposure time}$$

$$\times \left(\frac{\text{old N. A.}}{\text{new N. A.}}\right)^2 = 1/10 \left(\frac{0.25}{0.50}\right)^2 = 1/40 \text{ or,}$$

say, 1/50 second.

It is seen that more light reaches the photographic film with higher numerical apertures at the same magnification.

3 Film. Exposure time varies inversely with the American Standards Association speed index of the film.

Example: A good picture was obtained with Eastman Tri-X film at 1/100 second. What is the correct exposure for Eastman Kodachrome II Type A. The A. S. A. speed for Tri-X is 400 and for Kodachrome II Type A Professional is 40.

$$\text{new exposure time} = \text{old exposure time}$$

$$\times \frac{\text{A. S. A. of old film}}{\text{A. S. A. of new film}} = 1/100(400/40) =$$

10/100 or 1/10 second.

4 Other parameters may be varied but the prediction of exposure time cannot be made readily. Experience and photoelectric devices are the best guides to the proper exposure.

Photoelectric devices are excellent for determining correct exposure. Since ordinary photographic exposure meters are not sensitive enough for photomicrography, more sensitive instruments, having a galvanometer or electronic amplifying circuit, are required. Some photosensitive cells are inserted in the body tube in place of the eyepiece for light intensity readings. This has the advantage of detecting the light level at a point of high intensity but does not take into account the eyepiece, the distance to the film or the film speed.

The cell may be placed just above the eyepiece so that it registers the total amount of light leaving the eyepiece. Again, the effects of film speed and the projection distance are not accounted for. The principal drawback with the total light measuring photometer is the difficulty of taking into account the area of field covered. Take, for example, a bright field in which only a few crystals appear; perhaps 1 percent of the light entering the field of view is scattered by the crystals and the photometer shows close to a maximum reading. Now assume that everything remains constant except the number of crystals and, consequently, the amount of light scattered. The photometer reading could easily drop by 50 percent, yet the proper exposure is unchanged. The situation is similar for photomicrography with crossed polars since the photometer reading depends on the intensity of illumination, on the birefringence and thickness of the crystals and on the number and size of the crystals in the field or, alternatively, on the area of the field covered by birefringent crystals. One of the best solutions to this problem is to measure the photometer reading with no preparation on the stage. A first-order red compensator or a quartz wedge is inserted when crossed polars are being used to illuminate the entire field.

An alternative is to place the cell on the ground glass where the film will be located. However, although all variables except film speed are now taken into account, measurements in the image plane have the disadvantage of requiring a more sensitive electronic photoelectric apparatus.

No matter what method is used for placing the photocell, the exposure time can be determined by the general formula:

$$\text{exposure time} = \frac{k}{\text{meter reading}}.$$

The constant k will depend on the physical arrangement and film used. To determine k for any particular system, first set up the microscope to take a picture. Record the meter reading and take a series of trial exposures. Pick out the best exposure and calculate k. Then the k which was determined holds as long as no change is made in the light path beyond the photocell, e.g. changing to a faster film or changing the projection distance. Thus the objective, condenser position or illuminator may be changed without affecting k if the cell is used as described above.

Example: With one particular arrangement of photocell and film, the meter reading is found to be 40. A series of photographs are taken at 1/2, 1/5, 1/10, 1/25 and 1/50 seconds. The photomicrograph taken at 1/5 second is judged to be the best; hence k is calculated as follows:

k = meter reading × exposure time = $40 \times 1/5 = 8$.

Assume now that a new picture is to be taken at another magnification (but with the same film and projection distance) and that the new meter reading is 16; therefore:

exposure time = k/meter reading = $8/16 = 1/2$ second.

V MICROMETRY

A Particle Size Determination

Linear distances and areas can be measured with the microscope. This permits determination of particle size and quantitative analysis of physical mixtures. The usual unit of length for microscopical measurements is the micron (1×10^{-3}mm or about 4×10^{-5}inch). Measuring particles in electron microscopy requires an even smaller unit, the millimicron (1×10^{-3} micron or 10 Angstrom units). Table 6 shows the approximate average size of a few common airborne materials.

Table 6. APPROXIMATE PARTICLE SIZE OF SEVERAL COMMON PARTICULATES

Ragweed pollen	25 microns
Fog droplets	20 microns
Power plant flyash (after precipitators)	2-5 microns
Tobacco smoke	0.2 micron (200 millimicrons)
Foundry fumes	0.1 - 1 micron (100-1000 millimicrons)

The practical lower limit of accurate particle size measurement with the light microscope is about 0.5 micron. The measurement of a particle smaller than this with the light microscope leads to errors which, under the best circumstances, increase to about ± 100 percent (usually +).

One of the principal uses of high resolving power is in the precise measurement of particle size. There are, however, a variety of approximate and useful procedures as well.

1 Methods of particle size measurement

a Knowing the magnification of the microscope (product of the magnification of objective and eyepiece), the size of particles can be estimated. For example, with a 10X eyepiece and a 16-mm (or 10X) objective, the total magnification is 100X. A particle that appears to be 10-mm at 10 inches from the eye has an actual size of 10 mm divided by 100 or 0.10 mm or 100 microns. This is in no sense an accurate method, but it does permit quick estimation of particle size; the error in this estimation is usually 10-25 percent.

b Another approximate method is also based on the use of known data. If we know approximately the diameter of the microscope field, we can estimate the percentage of the diameter occupied by the object to be measured and calculate from these figures the approximate size of the object. The size of the microscope field depends on both the objective and the ocular although the latter is a minor influence. The size of the field should be determined with a millimeter scale for each objective and ocular. If this is done, estimation of sizes by comparison with the entire field diameter can be quite accurate (5-10%).

c The movement of a graduated mechanical stage can also be used for rough measurement of diameters of large particles. Stages are usually graduated (with vernier) to read to 0.1 millimeter, or 100 microns. In practice, the leading edge of the particle is brought to one of the lines of the cross hair in the eyepiece and a reading is taken of the stage position. Then the particle is moved across the field by moving the mechanical stage in an appropriate direction until the second trailing edge just touches the cross-hair line. A second reading is taken and the difference in the two readings is the distance moved or the size of the particle. This method is especially useful when the particle is larger than the field, or when the optics give a distorted image near the edge of the field.

d The above method can be extended to projection or photography. The image of the particles can be projected on a screen with a suitable light source or they may be photographed. The final magnification, M, on the projection surface (or film plane) is given approximately by

$$M = D \times O.M. \times E.M. / 25$$

where O. M. = objective magnification
E. M. = eyepiece magnification
D = projection distance from the eyepiece in centimeters.

The image detail can then be measured in centimeters and the actual size computed by dividing by M. This method is usually accurate to within 2-5 percent depending on the size range of the detail measured.

e The stated magnifications and/or focal lengths of the microscope optics are nominal and vary a bit from objective to objective or eyepiece to eyepiece. To obtain accurate measurements, a stage micrometer is used to calibrate each combination of eyepiece and objective. The stage micrometer is a glass microscope slide that has, accurately engraved in the center, a scale, usually 2 millimeters long, divided into 200 parts, each part representing 0.01 millimeter. Thus when this scale is observed, projected or photographed, the exact image magnification can be determined. For example, if 5 spaces of the stage micrometer measure 6 millimeters when projected, the actual magnification is

$$\frac{6}{5\ (0.01)} = 120 \text{ times.}$$

This magnification figure can be used to improve the accuracy of method 4 above.

f The simplest procedure and the most accurate is based on the use of a micrometer eyepiece. Since the eyepiece magnifies a real image from the objective, it is possible to place a transparent scale in the same plane as the image from the objective and thus have a scale superimposed over the image. This is done by first placing an eyepiece micrometer scale disc in the eyepiece. The eyepiece micrometer has an arbitrary scale and must be calibrated with each objective used. The simplest way to do this is to place the stage micrometer on the stage and note a convenient whole number of eyepiece micrometer divisions. The value in microns for each eyepiece micrometer division is then easily computed. When the stage micrometer is removed and replaced by the specimen, the superimposed eyepiece scale can be used for accurate measurement of any feature in the specimen by direct observation, photography or projection.

2 Calibration of eyepiece micrometer

Each micrometer stage scale has divisions 100μ (0.1 mm) apart; one or two of these are usually subdivided into 10μ (0.01-mm) divisions. These form the standard against which the arbitrary divisions in the micrometer eyepiece are to be calibrated. Each objective must be calibrated separately by noting the correspondence between the stage scale and the eyepiece scale. Starting with the lowest power objective focus on the stage scale, arrange the two scales parallel and in good focus. It should be possible to determine the number of eyepiece divisions exactly equal to some whole number of divisions of the stage scale, a distance readily expressed in microns.

The calibration consists, then, of calculating the number of microns per eyepiece scale division. To make the comparison as accurate as possible, a large part of each scale must be used (see Figure 17). Let's assume that with the low power 16-mm objective 6 large divisions of the stage scale (s. m. d.) are equal to 38 divisions of the eyepiece scale. This means that 38 eyepiece micrometer divisions (e. m. d.) are equivalent to 600 microns. Hence:

$$\begin{aligned} 1 \text{ e. m. d.} &= 600/38 \\ &= 15.8\mu. \end{aligned}$$

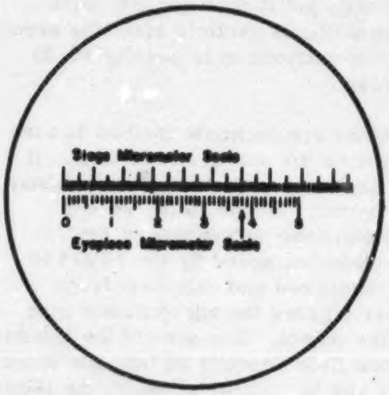


Figure 17

COMPARISON OF STAGE MICROMETER SCALE WITH EYEPIECE MICROMETER SCALE

Thus when that micrometer eyepiece is used with that 16-mm objective each division of the eyepiece scale is equivalent to 15.8μ, and it can be used to make an accurate measurement of any object on the microscope stage. A particle, for example, observed with the 16-mm objective and measuring 8.5 divisions on the eyepiece scale is 8.5 (15.8) or 135μ in diameter.

Each objective on your microscope must be calibrated in this manner.

A convenient way to record the necessary data and to calculate μ/emd is by means of a table.

Table 7

Objective	No. smd = no. emd	μ = no. emd	μ = 1 emd
32-mm	18 = 44	1800 = 44	40. 9μ
16-mm	6 = 38	600 = 38	15. 8μ
4-mm	1 = 30	100 = 30	3. 33μ

3 Determination of particle size distribution

The measurement of particle size can vary in complexity depending on particle shape. The size of a sphere may be denoted by its diameter. The size of a cube may be expressed by the length of an edge or diagonal. Beyond these two configurations, the particle "size" must include information about the shape of the particle in question, and the expression of this shape takes a more complicated form.

Martin's diameter is the simplest means of measuring and expressing the diameters of irregular particles and is sufficiently accurate when averaged for a large number of particles. In this method, the horizontal or east-west dimension of each particle which divides the projected area into halves is taken as Martin's diameter (Figure 18).

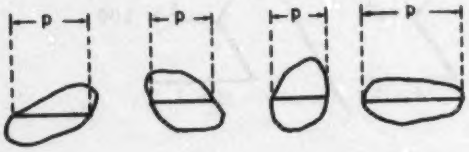


Figure 18

MARTIN'S DIAMETER

The more particles counted, the more accurate will be the average particle size. Platelike and needlelike particles should have a correction factor applied to account for the third dimension since all such particles are restricted in their orientation on the microscope slide. When particle size is reported, the general shape of the particles as well as the method used to determine the "diameter" should be noted.

Particle size distribution is determined routinely by moving a preparation of particles past an eyepiece micrometer scale in such a way that their Martin's diameter can be tallied. All particles whose centers fall within two fixed divisions on the scale are tallied. Movement of the preparation is usually accomplished by means of a mechanical stage but may be carried out by rotation of an off-center rotating stage. A sample tabulation appears in Table 8. The eyepiece and objective are chosen so that at least six, but not more than twelve, size classes are required and sufficient particles are counted to give a smooth curve. The actual number tallied (200 - 2,000) depends on particle shape regularity and the range of sizes. The size tallied for each particle is that number of eyepiece micrometer divisions most closely approximating Martin's diameter for that particle.

4 Calculation of size averages

The size data may be treated in a variety of ways; one simple, straightforward treatment is shown in Table 9. For a more complete discussion of the treatment of particle size data see Chamot and Mason's *Handbook of Chemical Microscopy*(3), page 26.

The averages with respect to number, $\bar{d}_1$; surface, $\bar{d}_3$; and weight or volume, d_4, are calculated as follows for the data in Table 9.

Table 8. PARTICLE SIZE TALLY FOR A SAMPLE OF STARCH GRAINS

Size class (emd*)	Number of particles	Total
1	𝍸 𝍸 𝍸 |	16
2	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 | | |	98
3	𝍸 𝍸	110
4	𝍸 | |	107
5	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 |	71
6	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸	45
7	𝍸 𝍸 𝍸 𝍸 |	21
8	| |	2
		470

*emd = eyepiece micrometer divisions

$\overline{d}_1 = \Sigma nd/\Sigma n = 1758/470$

$= 3.74 \text{ emd} \times 2.82^* = 10.5\mu$

$\overline{d}_3 = \Sigma nd^3/\Sigma nd^2 = 37440/7662$

$= 4.89 \text{ emd} \times 2.82 = 13.8\mu$

$\overline{d}_4 = \Sigma nd^4/\Sigma nd^3 = 199194/37440$

$= 5.32 \text{ emd} \times 2.82 = 15.0\mu$

*2.82 microns per emd (determined by calibration of the eyepiece-objective combination used for the determination).

Cumulative percents by number, surface and weight (or volume) may be plotted from the data in Table 9. The calculated percentages, e.g.

$$\sum_{d=1}^{d} nd^4 \bigg/ \sum_{d=1}^{d=15} nd^4 \times 100$$

for the cumulative weight or volume curve, are plotted against d. Finally, the specific surface, S_m, in square meters per gram, m, may be calculated if the density, D, is known; the surface average $\overline{d}_3$, is used.

If $D = 1.1$, $S_m = 6/\overline{d}_3 D = 6/13.8(1.1)$

$= 0.395 m^2/g.$

Table 9. CALCULATIONS FOR PARTICLE SIZE AVERAGE

d (Aver. diam. in emd)	n	nd	nd^2	nd^3	nd^4
1	16	16	16	16	16
2	98	196	392	784	1568
3	110	330	990	2970	8910
4	107	428	1712	6848	27392
5	71	355	1775	8875	44375
6	45	270	1620	9720	58320
7	21	147	1029	7203	50421
8	2	16	128	1024	8192
	470	1758	7662	37440	199194

B Counting Analysis

Mixtures of particulates can often be quantitatively analyzed by counting the total number of particulates from each component in a representative sample. The calculations are, however, complicated by three factors: average particle size, particle shape and the density of the components. If all of the components were equivalent in particle size, shape and density then the weight percentage would be identical to the number percentage. Usually, however, it is necessary to determine correction factors to account for the differences.

When properly applied, this method can be accurate to within ± 1 percent and, in special cases, even better. It is often applied to the analysis of fiber mixtures and is then usually called a dot-count because the tally of fibers is kept as the preparation is moved past a point or dot in the eyepiece.

A variety of methods can be used to simplify recognition of the different components. These include chemical stains or dyes and enhancement of optical differences such as refractive indices, dispersion or color. Often, however, one relies on the differences in morphology, e.g. counting the percent of rayon fibers in a sample of "silk".

Example 1: A dot-count of a mixture of fiberglass and nylon shows:

nylon	262
fiberglass	168

Therefore:

$$\% \text{ nylon} = 262/(262 + 168) \times 100$$
$$= 60.9\% \text{ by number.}$$

However, although both fibers are smooth cylinders, they do have different densities and usually different diameters. To correct for diameter one must measure the average diameter of each type of fiber and calculate the volume of a unit length of each.

	aver. diam. μ	volume of $1\text{-}\mu$ slice, μ^3
nylon	18.5	268
fiberglass	13.2	117

The percent by volume is, then:

$$\% \text{ nylon} = \frac{262 \times 268}{(262 \times 268)+(168 \times 117)} \times 100$$
$$= 78.1\% \text{ by volume.}$$

Still we must take into account the density of each in order to calculate the weight percent.

If the densities are 1.6 for nylon and 2.2 for glass then the percent by weight is:

$$\%\ \text{nylon} = \frac{262 \times 268 \times 1.6}{(262 \times 268 \times 1.6) + (168 \times 117 \times 2.2)} \times 100$$

$$= 72\%\ \text{by weight.}$$

Example 2: A count of quartz and gypsum shows:

quartz	283
gypsum	467

To calculate the percent by weight we must take into account the average particle size, the shape and the density of each.

The average particle size with respect to weight, $\bar{d}_4$, must be measured for each and the shape factor must be determined. Since gypsum is more platelike than quartz each particle of gypsum is thinner. The shape factor can be approximated or can be roughly calculated by measuring the actual thickness of a number of particles. We might find, for example, that gypsum particles average 80% of the volume of the average quartz particle; this is our shape factor. The final equation for the weight percent is:

$$\%\ \text{quartz} = \frac{283 \times \pi\bar{d}_4/6 \times Dq}{238 \times \pi\bar{d}_4/6 \times Dq + 467 \times \pi\,\bar{d}_4^*/6 \times 0.80 \times Dg} \times 100$$

where Dq and Dg are the densities of quartz and gypsum respectively; 0.80 is the shape factor and $\bar{d}_4$ and $\bar{d}_4^*$ are the average particle sizes with respect to weight for quartz and gypsum respectively.

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DESCRIPTORS: Microscope and Optical properties

ARTIFICIAL SUBSTRATES

I INTRODUCTION: THE NATURE OF ARTIFICIAL SUBSTRATES

A Artificial substrates are anything deliberately placed in the water for the purpose of providing a place for benthic or attached (sessile, sedentary, etc.) organisms to grow on or in. This is in contrast to "bait" which is used as an attractant.

B Their origins for commercial use, or human food production are rooted in antiquity. Some examples are:

1 Ropes, poles, brush, concrete structures, and other objects thrust into the bottom, or suspended in estuarine waters to catch and grow oysters and mussels (cultural techniques), known virtually around the world.

2 Straw or reed tepees planted in shallow alkaline lakes (in Mexico for example) to catch the eggs of Corixids (Insecta: Order Hemiptera, back-swimmers). Eggs are harvested by drying and brushing them off onto white sheets. Used for human food.

C The fouling of ships bottoms, piling, etc. by barnacles and other marine life is an "artificial substrate in reverse".

D The use of aggregate to support a zoogloeal mass of micro-biota in a trickling filter, thus simulating a riffle area in a surface stream, is a modern concept to harness and make use of "consumer" and "reducer" elements of a community in order to dissipate the energy (oxidize, exhaust the food value) contained in sewage.

II ECOLOGICAL BASIS

A Artificial substrates are based on the "laws of organismal distribution."

1 Any given kind of organisms tends to be present (inhabit) in all available suitable habitats.

2 Any given habitat tends to be inhabited by all suitably adapted kinds of organisms.

B A "substrate" being an object (or group of objects) constitutes a habitat suitable for sessile or attached organisms, and also those that naturally burrow in, crawl over, or otherwise live associated with objects. Natural objects here could mean the bottom, stones, sticks (floating or sunk), etc.

C Organisms that would not be attracted to substrates would be plankton and nekton (fish and larger swimming invertebrates).

D Ecological Succession

Colonization is rapid in a biologically productive water, and normally reaches a stable climax* community in about a month. A typical outline of successive forms to appear in a freshwater situation, for example, might be as follows.

1 Periphyton (slime forming) stage (see also below)

a Bacteria - within an hour

b Diatoms - within the first day

c Other micro-algae - within the first day

d Protozoa - within the first day

2 Macroinvertebrate dominated stage (see also below)

a Primary attached or sedentary colonizers - second to third day

1) Net caddisflies

2) Bryozoa

3) Cordylophora caspia

4) Hydra

---

*A community which has achieved a point of no further change, under a given set of environmental conditions. Time scale may vary with circumstances.

NOTE: Mention of commercial products and manufacturers does not imply endorsement by the Environmental Protection Agency.

b Primary foragers

1) Mayflies

2) Stoneflies

3) Midges

c Secondary attached or sedentary colonizers:

1) Sponges

2) Filamentous algae

d Adventitious forms

1) Crustaceans

2) Flatworms

3) Leeches

4) Snails

5) Other

3 Artificial substrates in a marine environment proceed through similar stages, except that the macroinvertebrate stage may be more subject to variation in the attachment of broods of barnacle, oyster, and other larvae resulting from greater numbers of types present, tidal current variation, meteorological conditions, etc.

III ARTIFICIAL SUBSTRATES AS SCIENTIFIC COLLECTING DEVICES

A A review of the history of artificial substrates for collecting microorganisms (aufwuchs) (Cooke, 1956) indicates that glass microscope slides were first used for this purpose about 1915. Wood or metal panels appear, however, to have been deliberately exposed for the scientific collection of larger organisms at least since approximately the turn of the century, and probably long before that (Visscher, 1928).

B Biological Applications

The principles of the artificial substrate remain the same, regardless of the community sampled. Two general types of communities and associated samplers have been employed:

1 Periphyton (or aufwuchs) samplers

Periphyton is the community of slime forming microorganisms which is the first to attach to objects newly exposed under water. This community is generally considered to provide an anchor layer to which other higher forms of life can more readily attach. It tends to persist until overgrown or displaced by larger organisms, and then in turn can be found spreading over the surfaces of these same larger plants and animals.

2 Periphyton has been widely studied as it appears on 1 × 3 glass microscope slides which are equally convenient to expose in the field and to study in the laboratory.

3 Particular studies have included:

a The original bacterial and fungal slime

b Diatom identification and counts

c Identification and counts of other microscopic algae

d Protozoans

e Primary productivity

4 The macroinvertebrate community is sampled by a great variety of devices such as those cited below. The organisms are usually removed from the substrate for study. Applications have included the following:

a General study of the macroinvertebrate community b Estimates of productivity

c Studies of the life cycle of particular species

d Studies of the influence of the substrate on the attachment of sessile forms

1) The influence of toxic paints for the prevention of fouling organisms

2) Wood panels to study the penetration of boring molluscs and crustaceans

C Effect of type of device on what is collected

1 Wood boring organisms like teredo worms (Mollusca, Pelecypoda) or gribbles (Arthropoda, Isopoda) would obviously be attracted primarily to wood (although some are known to bore in other materials).

2 Delicate forms and crawling forms would be most likely to be collected on devices having a shape to protect against strong currents.

3 Those with strong attachments could endure swift currents; often, surprisingly, even during periods of original attachment (ex. byssus attached clams which are also benthic forms).

4 Bottom burrowers would be most likely collected in artificially contained portions of bottom material.

D Effect of Location

1 The depth at which a sampler is suspended may influence the organisms attracted.

2 Location in or out of a current, direct sunlight, etc., will influence the take.

E Some Types of Devices

1 Cement plates, panels, and blocks

2 Ceramic tiles

3 Wood blocks

4 Metal plates

5 Glass slides - 1 × 3 inch micro slides are used by many workers. Numerous devices are employed to hold them. They are generally either floated (Weber and Raschke 1966) or suspended in racks, anchored to submerged bricks or other objects.

6 Plastic petri dishes

Burbanck and Spoon utilized an ordinary 50 × 12 mm plastic petri dish for collecting sessile protozoa. Sickle modified this by using a styrofoam cup (6 oz. size) with the bottom third being cut off. The lower unit of the plastic dish is easily wedged into place in the cup and the device is simply held by a nylon line on a rope held in place by an appropriate anchor and float.

The cup which tends to float is so held that the petri dish bottom is in a horizontal position and bottom side up.

7 Multiple plate (Hester and Dendy, 1962)

a Common current procedure utilizes 3-inch squares of 1/4 inch thick Masonite separated by 1-inch square spacers.

These may be:

b Threaded on an eye bolt or long rod.

c Suspended by a loop of nylon cord.

8 Baskets or trays of bottom-type material

a Trays of bottom material sunk in the surface layer of the bottom.

b Baskets of stones suspended in the water (Anderson and Mason, 1966).

9 Boxes, cages, bundles, etc., of brush, reeds, or artificial material.

10 Polyethylene tapes

11 Plastic webbing

Minnesota Mining and Manufacturing Company conservation web no. 200.

12 Styrofoam

13 Glass coverslips

Small slips are floated on the surface of the water. Highly useful for protozoa and rotifers. Remove and place on a micro slide. Examine as a wet mount.

F Retrieval is an acute problem with all of these samplers.

1 Physical factors

a Relocation

b Floods and drift

c High water

2 Well marked samplers or floats are naturally vulnerable to the public, resulting in disturbed, damaged, or destroyed sample gear.

a This has been overcome by an ingenious submerged float and recovery line device. The weak link in a submerged recovery line is a modified flash bulb. An electronic device actuated by an underwater gun breaks the bulb allowing the float and attached line to surface. (Ziebell, McConnell, and Baldwin)

b This unit has been further modified by Fox (University of Georgia Cooperative Fishery Unit) who used an inexpensive detonator, "Seal Salute". The latter is an inexpensive fused charge designed for underwater explosion.

IV ARTIFICIAL SUBSTRATES OR SAMPLERS, AND WATER QUALITY

A Artificial substrates provide a habitat ("place to live"). It follows from the laws of distribution (II A 1 and 2 above), that the community which inhabits a device will be governed by the physical nature or structure interacting with the characteristics of the surrounding water (velocity, temperature, chemical characteristics, etc.). Since the nature of the sampler is controlled, it is evident that the characteristics of the water constitute the variable factor.

B Water Quality Surveillance

1 Similar substrates suspended side by side in the same water tend to accumulate (essentially) the same communities and quantities of organisms.

2 Similar substrates suspended in different waters accumulate different communities and quantities.

3 Ergo: different communities and quantities collected from similar substrates at different places and times, probably indicate different water qualities.

a These may be natural (seasonal, diurnal, etc.)

b Or they may be a result of human influences (pollution)

c A series of samplers the length of a stream, lake, or estuary can suggest "steady state" differences in water quality.

d A series of samplers exposed over a period of time at a given site can suggest changes of water quality in time.

4 The artificial substrate thus essentially constitutes an in-situ bioassay of the water.

C Interpretation and Significance of Collections

1 The unit of comparison is most appropriately taken as "the sampler". The artificial substrate by definition is not the natural local bottom material, and unless it consists of a portion of that bottom which has been actually removed and replaced in an artificial container (III-D-7) the composition and magnitude of the community it contains may or may not bear a definitive relationship to the actual natural problem. The take of the artificial substrate thus may have relatively little relationship to the take of a Petersen or an Ekman grab (dredge).

2 Comparisons between different types of samplers are likewise hazardous. Each is what it is, and if they are different they are not identical; thus the biota each collects cannot be expected to be identical (CF: II A).

3 Artificial substrates should generally be compared on a "sampler vs sampler" basis, or for periphyton, "unit area vs unit area".

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DESCRIPTORS: Artificial Substrates, Aquatic Life, Benthos, Bottom Sampling, and Invertebrates.

BENTHIC INTEGRITY AND MACRO INVERTEBRATE DRIFT

I Significant number of benthic macro invertebrates under certain conditions join stream drift.

A This phenomenon was only discovered in mid century. The organisms as well as the phenomenon is termed collectively, Drift.

B Macroinvertebrates which drift include are the insect orders, Ephemeroptera, Trichoptera, Plecoptera and the crustacean order Amphipoda.

C Other invertebrate groups exhibit drift patterns.

II FOUR BASIC TYPES OF DRIFT ARE RECOGNIZED

A Catastrophic Drift

Floods wash numerous benthic organisms downstream. Application of pesticides may also cause such drift.

B Constant Drift (Incidental or Adventitious)

Organisms are constatly being dislodged from the substrate during normal activities and carried downstream.

C Periodic (Diel) Drift

In contrast to the other categories, this is a specific behavior pattern and related to circadian activity rhythms. Periodic or diel drift occurs in peaks for successive 24-hour periods.

1 Night-active. Light intensity is the phase-setting mechanism.

2 Day-active. Water temperature appears to be the phase-setter.

D Seasonal Drift (Related to Life History Development)

Seasonal drift occurs, for example, in some maturing stoneflies which drift downstream for emergence. This is another reason for a serious consideration of drift in bottom fauna sampling since such presence of stoneflies could easily be misinterpreted.

III DIEL DRIFT

A Diel activity rhythms generally include two peaks during the 24-hour period; one major and the other minor.

1 The bigeminus type in which the major peak occurs first (after sunset).

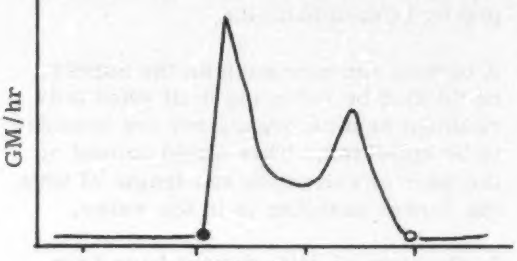

FIGURE 1

2 The alternans pattern with the major peak occurring last.

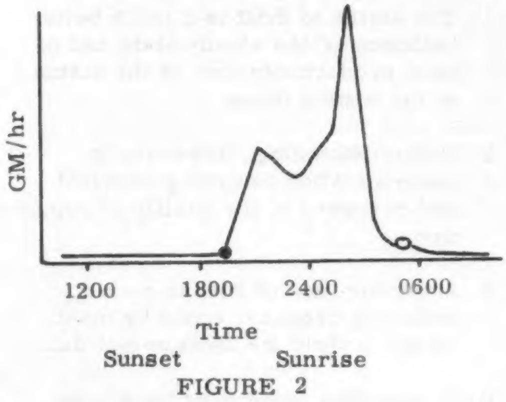

FIGURE 2

B Drift Rate and Density (Waters, 1969)

1 Drift rate defined is "...the quantity of organisms passing a width transect or portion thereof, per unit time;

it is a measure of displacement or the movement of organisms from one place to another."

2 Drift density"... is the quantity of organisms per unit volume of water, in much the same way as plankton density can be defined."

IV DETERMINING BENTHIC INTEGRITY THRU DRIFT SAMPLING

A The drift from productive upstream reaches may support a fish population existing in relatively barren stream sections.

B Drift will colonize artificial substrates, such as suspended rock baskets, when placed in such habitats.

C A bottom sampler such as the Surber, could also be sampling drift when only resident benthic organisms are intended to be collected. This would depend on the hour of collection and length of time the Surber sampler is in the water.

D Application of drift studies have been widely used in pesticide related studies and routine monitoring. Dimond concluded that:

1 The status of drift is a much better indicator of the steady state and of total productivity than is the status of the bottom fauna.

2 Bottom sampling, however, is superior when analyzing survival and recovery of the quality of population.

3 A combination of both in such a sampling program would be most likely to yield the most useful data.

E Drift sampling techniques have been useful for recovery of large numbers of sand-dwelling mayflies, which were once rarely collected.

V MAJOR TAXA INVOLVED IN DRIFT

A The crustacean order Amphipoda

1 Gammarus species

2 Hyalella azteca

B The Insect Orders

1 Ephemeroptera

Baetis species (apparently universal)

2 Plecoptera

3 Trichoptera

4 Diptera
Simuliidae

5 Elmidae

C The main groups exhibiting very high drift rates include: Baetis, some Gammarus species, and some Simuliidae.

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DESCRIPTORS: Aquatic Life, Aquatic Drift, and Invertebrates

EFFECTS OF THERMAL POLLUTION ON THE BENTHOS

I INTRODUCTION

A Fish may be obviously important but they are not the only important organisms or the only ones being studied at present. The food organisms are just as important in the long run. Lose the organisms which convert the energy of the primary producers to a form usable by the fish and we lose the fish themselves.

B In a river, most of the microscopic population are benthic organisms, since the plankton have difficulty maintaining position in the stream flow. The benthos is a stationary community which should reflect the action of the temperature in the area of influence. Of course, bottom debris may serve to protect benthic organisms to some extent from full exposure to the heated water.

II SUBLETHAL EFFECTS ON AQUATIC INSECTS

A In most western streams the stoneflies, caddisflies and mayflies are the primary fish food organisms. At the same time, these organisms have definite environmental requirements and cold, well-oxygenated water is a prime factor.

1 Preliminary work at the Duluth Laboratory indicates that temperatures would probably become lethal to any cold water fish like trout before the insects would die.

a According to Usinger (1956), the heat tolerance of macroscopic invertebrates is well above that of fish.

b For example, soldier fly (stratiomyidae) larvae were found living in thermal waters at temperatures up to 120°F.

2 Table 1 shows 96 hour TL_m values determined for some insect species by Nebeker and Lemke of the Duluth Laboratory.

a These temperatures are well above the 12°C suggested as the maximum limit for spawning and egg development in salmon and trout.

b This doesn't tell the whole story because the insects may be harmed in other ways.

B Gaufin, formerly of Utah, and also Nebeker of the Duluth Laboratory, have demonstrated that temperature increases can cause premature emergence.

1 A 10°C rise from ambient winter temperature caused one species of stonefly to emerge in January instead of May. "One must imagine how perplexed these organisms must be as they expect nice warm spring weather only to freeze to death as they emerge."

2 Nebeker found that a temperature increase for another species caused the males to emerge as much as two months ahead of the females!

C Either situation would prevent reproduction and would be fatal to the species although not fatal to individuals prior to emergence.

D Even without lethal effects we may find changes in community due to variation in optimum temperatures between species. This has not been studied enough in the field to really determine the overall effect on a natural system but it is something which we will have to know more about in the future.

III SUBLETHAL EFFECTS ON SHELLFISH

A Most shellfish, such as clams, oysters, crabs and lobsters, which are directly beneficial to man as a food source, are marine, stenothermal organisms. Some species are stenothermal for one developmental stage and eurythermal for another. Generally, however, breeding and spawning requirements are stenothermal.

1 The time of mollusc, e. g. clams, oyster, etc., spawning is temperature dependent.

2 Most molluscs with specific temperature breeding relationships spawn in the spring and summer, and many do not spawn until a certain temperature is reached.

B The American oyster Crassostrea virginica spawns at temperatures between 15 and 34°C

TABLE 1

Temperatures at which 50% of the test species died after 96 hours exposure (TL_m^{96}) when acclimated at 10°C for one week.

Species Tested	TL_m^{96} (°Celcius)
Taeniopteryx maura (winter stonefly)	21°
Ephemerella subvaria (mayfly)	21.5°
Isogenus frontalis (stonefly)	22.5°
Allocapnia granulata (winter stonefly)	23°
Stenonema tripunctatum (mayfly)	25.5°
Brachycentrus americanus (caddisfly)	29°
Pteronarcys dorsata (stonefly)	29.5°
Acroneuria lycorias (stonefly)	30°
Paragnetina media (stonefly)	30.5°
Atherix variegata (true fly)	32°
Boyeria vinosa (dragonfly)	32.5°
Ophiogomphus rupinsulensis (dragonfly)	33°

12°C (55°F) Maximum temperature recommended in Water Quality Criteria for spawning and egg development of salmon and trout.

From: Nebeker, Alan V. and Armond E. Lemke, 1968. Preliminary studies on the tolerance of aquatic insects in heated waters. Journal of Kansas Entomological Society 41: 413-418. July, 1968.

(59 and 93.2°F) depending on its condition, and spawning is usually triggered by a rise in temperature.

C Many species tolerate temperatures in excess of those at which breeding occurs.

  1 For example, the shore crab Carcinus maenas thrives, but does not breed, at temperatures of 14 to 28°C (57.2 to 82.4°F).

  2 In this case, temperature limits the population, but migration of organisms can occur from outside the heated area.

D Physiology, metabolism and development are all affected by temperature.

  1 The American oyster C. virginica ceases feeding at temperatures below 7°C (44.6°F).

    a Above 32°C (89.6°F) ciliary activity, which is responsible for water movement, is decreased.

    b At 42°C (107.6°F) almost all body functions cease, or are reduced to a minimum.

  2 The European oyster Ostrea lurida tends to close its shell as temperatures drop.

    a At 4 to 6°C (39.2 to 42.8°F) the oyster's shell remains closed most of the time.

    b At 6 to 8°C (42.8 to 46.4°F) the shell opens for about 6 hours per day.

    c At 15°C (59°F) the shell stays open for 23 hours a day.

E Very little is known about prolonged effects of temperatures above 32 to 34°C (90 to 94°F) on oysters; however, long exposure to such temperatures may impede the oyster's normal rate of water circulation. When either low or high temperatures cause shells to close or ciliary action to cease, oysters cannot feed and subsequently lose weight. Thus, temperature changes can produce an effect similar to chronic toxicity.

F The distribution of benthic organisms is temperature dependent.

  1 The American oyster C. virginica is present in Gulf Coast waters that may vary between 4 and 34°C (39.2 and 93.2°F), but the European oyster O. edulis is restricted to water temperatures of 0 to 20°C (32 to 68°F).

  2 The opossum shrimp Neomysis americana is not often found at temperatures above 31°C (87.8°F) in the Chesapeake estuary.

IV LETHAL EFFECTS

A Studies of particular species of benthic macroinvertebrates have indicated that lethal temperatures vary considerably with the type of organism.

  1 Laboratory investigations on the freshwater snail Lymnaea stagnalis showed a lethal temperature of 30.5°C (89.6°F), while the species Viviparous malleatus did not succumb until the temperature reached 37.5°C (99.5°F).

  2 Agerborg (1932) observed a freshwater snail, Physa gyrina, living and reproducing nicely in zones up to 91.4°F in heated wastewater.

  3 Hutchinson (1947) reported that Viviparous malleatus, a freshwater snail, was not killed until the temperature reached 37.5°C (99°F).

B Several snails, including Australorbis glabratus, suffered heat damage at 105.8°F (Von Brand, et. al. 1948).

C Other examples show that the limpet, Ancylus fluviatilus, was not hurt by a temperature of 96.8°F while 87.8°F was lethal to Acrolexus lacustris (Berg, 1952).

D When an unidentified species of crayfish was acclimated to 45°F, it had a lethal temperature of 93°F (Trembly 1961).

E Sprague (1963) reported a 24-hour lethal temperature of 94.3°F for a freshwater sowbug, Asellus intermedius, and a scud, Gammarus fasciatus. Another scud, Hyalella azteca, was killed at 91.8°F.

F Field work on rivers has indicated that benthic organisms decrease in number when water temperature exceeds 30°C (86°F).

  1 The macroinvertebrate riffle fauna of the Delaware River has decreased due to heated water discharges.

a At $35^{\circ}C$ ($95^{\circ}F$) many caddisfly, Hydropsyche, were dead, and those which remained alive were extremely sluggish.

b This study suggests that there is an upper tolerance level near $32.2^{\circ}C$ ($90^{\circ}F$) for a variety of different benthic forms with extensive losses in numbers and diversity accompanying a further increase in temperature.

V POPULATION SHIFTS CAUSED BY HEAT ADDITION

A Trembley (1960) studied the bottom fauna of the Delaware River at the Martins Creek Power Plant.

1 In the zone of maximum temperature rise rise just below the outfall, there was obvious reduction of species and individuals.

2 In the cool water unaffected by the thermal overflow, there was no reduction in macroinvertebrates.

3 During the cooler seasons there was repopulation of the areas affected during the hot months by thermal discharge.

4 Even during the summer, there was a significantly higher standing crop at the downstream site in comparison to the normal river control station.

B Coutant (1962) followed Trembley's Martins Creek research with a study of the macroinvertebrate bottom fauna of the riffle areas of Big Kaypush and Little Kaypush Rapids.

1 He confirmed Trembley's conclusions. From July through October, there was substantial reduction in the number, diversity, and biomass of benthic organisms in the path of the heated water.

2 At a distance of one mile downstream from the point of discharge, he found a normal population structure.

3 In his traverse studies, he observed an increase in both variety and number of organisms as he progressed from hot to cool water, demonstrating the effect of temperature as the primary limiting factor.

4 The work also showed the restricted effect of heated discharges in changing the biological communities. The data suggest a tolerance limit near $90^{\circ}F$ for a normal population structure with extensive loss in numbers and diversity of organisms accompanying further rise.

C Wurtz and Dolan (1960) reported a study on bottom organisms in the Schuylkill River at the Cromby Power Plant.

1 These authors gave no temperature data; however, the subcommittee of the Pennsylvania Electric Association (Mason, 1962) showed severe temperature alteration in this reach of river since the plant used 85% of the river flow as cooling water.

2 The river showed a very elevated temperature and slow recovery. Wurtz and Dolan evaluated the effects of heated discharges in terms of biological depression, biological distortion, and biological skewness.

3 Station 10 at Phoenixville Pumping Station, 0.5 miles below the plant, showed the greatest deviation.

4 At Station 13, six miles below the power plant, the river biology had recovered. This case illustrates ultimate recovery from an extreme condition.

VI SUMMARY

It is clear from the valid biological data presented that increased temperature of the water does alter the species and individual composition of the benthic population which, of course, being generally sessile, is unable to avoid exposure.

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